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CLINICAL AND EXPERIMENTAL

THE EFFECT OF BLOOD PRESSOR EPISODES ON BASOPHILIC AGGREGATION COUNTS*

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IN A previous report[1] we have shown that in normal individuals a physiologic rhythm occurs in regard to basophilic aggregation counts as was evidenced by the variations occurring from day to day. In the subjects studied this rhythm coincided with the daily meteorologic alterations, and the observed peaks, in most instances, were in accord with the points of high barometer and low temperature.

This is further exemplified in Fig. 1, which is a study made of a hospitalized male† afflicted with arteriolosclerosis and possibly periarteritis nodosa. Examination of the basophilic aggregation curve shows periods of increase which have been numbered 1 to 11 (no determinations February 7, 10, 12, 15) and from which stippled vertical columns have been carried up to the meteorograph. It is noted that episodes 1 to 5 all occur with polar infalls.‡ During the period when no counts were made a series of infalls occurred, but after the resumption of the counts we note that episodes 6 to 11 are again all associated with polar infalls. The maximum limits of variation are also noted and are from 750 to 10,000 basophilic aggregations per million red blood cells.

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†The patient is described in detail by Petersen as Vascular Subject 5 in Volume IV, *The Patient and the Weather.*

‡Polar Infall is the meteorologic designation for that period in which a mass of polar air is spreading over the earth's surface and is reflected in a rising barometer and a lowering of temperature.

Tropical Front indicates the sudden change from polar air to the typical warm moist air mass which revolves in a counterclockwise fashion, and which in America commonly travels from West to East. It is this rapidly moving air mass which characterizes the cyclonic circulation of the northern hemispheres, and has its reflection in a low barometer and a high temperature.

The association of basophilic aggregation peaks with polar episodes was explained on the basis of irritability of the bone marrow due to spasm of the capillaries and small blood vessels with a resulting anoxia as described in detail in Volume I, Part 2, of *The Patient and the Weather*. A similar explanation has been suggested by Rosegger[2] in a recent publication.

If this explanation is correct, the employment of a pressor active principle should stimulate reticulocyte formation and result in basophilic aggregation peaks higher than those existing as a result of natural occurring meteorologic alterations. With this in view, it was decided to perform experiments with

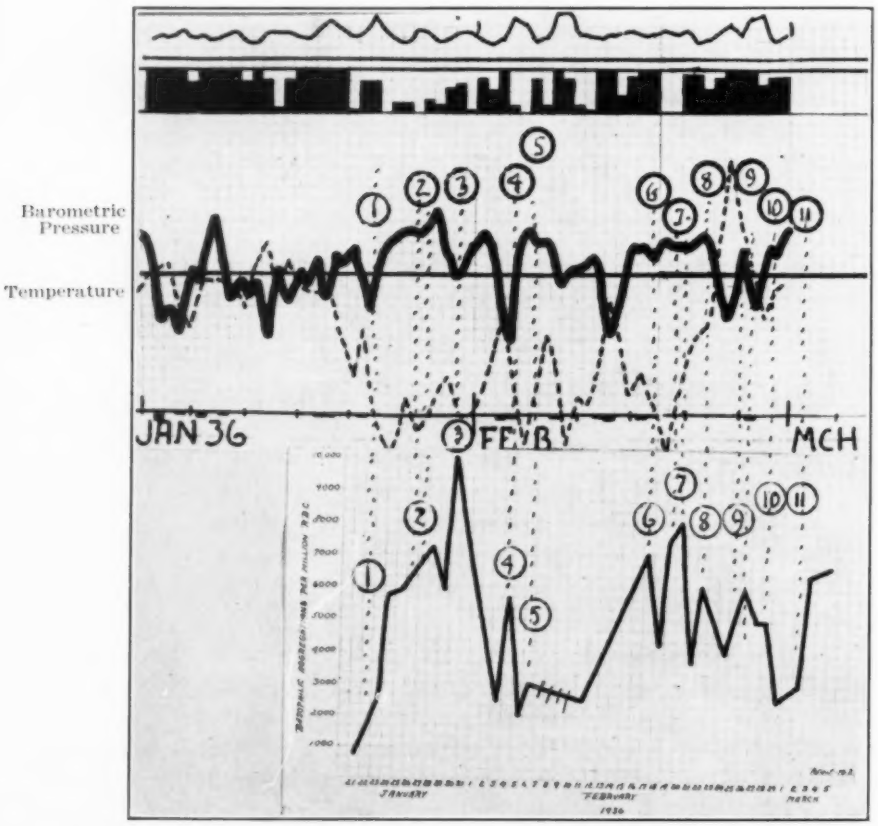

Fig. 1.—Basophilic aggregation counts in Vascular Subject 5 with superimposed meteorograph for the time under consideration. Polar infalls have been numbered 1 to 11 and the corresponding reaction of increase in basophilic aggregates is indicated. In the meteorograph the upper curve is the wind velocity, the black columns the percentage of cloudiness, the heavy line the barograph, and the dotted line the mean daily temperature.

rabbits, employing the pressor principle of the posterior lobe of the pituitary gland. In this manner a controlled method would be at hand whereby, artificially and at any selected time, we could produce a general organic status of spasm and tissue stimulation and irritability (ARS phase)* which, when the effect was produced in the bone marrow, would be analogous to that resulting with ordinary polar episodes of the cyclonic circulation.

*ARS phase is the time period during which tissues become stimulated, an acidity develops, metabolism increases, and tissue-stimulating substances are formed because of the relative anoxemia and the interference thereby caused in the course of normal metabolism.

EXPERIMENTS

Rabbits were chosen as the experimental animals and the two selected were observed first during the months of March, April, and May of 1935, and then during September and October of the same year. Two contrasting times of the year were selected in order to make note of seasonal variations in addition to daily variations. Pitressin (P. D. & Co.) was employed as the pressor agent. In each instance daily basophilic aggregation counts were made for several days before the utilization of the pitressin in order to determine normal daily variation. The McCord method of staining and counting was employed as in our first report. The pitressin was given intravenously in the marginal vein of the ear at the indicated intervals, and the dosage at each time of injection never exceeded 0.5 c.c.

In Fig. 2 are seen the observations made on Rabbits R^1 and R^2 during March, April, and May, 1935. The meteorograph of this period has been superimposed on the charts illustrating the reticulocyte counts. Verticle stippled lines have been carried through the graph on days that are followed by increases in reticulocytes. For the period preceding the injection of pitressin the crests of the curve are numbered 1 to 10, and for the period following pitressin 11 to 18. The synchronization is noted in Table I.

TABLE I

METEOROLOGIC EPISODES	DATE OF EPISODES	DATE OF BASOPHILIC AGGREGATION PEAKS		METEOROLOGIC ASSOCIATION
		RABBIT 1	RABBIT 2	
1 March	23	25		Beginning polar infall
2	25		27	Polar infall
3	27	29		Polar infall
4	29-31		29	Polar infall
5 April	1		1	Polar infall, irregular
6	2	2	3	
7	5	4	5	Polar infall
8	7-8	8	7	Polar infall
9	9	12-13	10	Barometric peak
10	11		12	Beginning polar infall
		After Pitressin		
11	15	15	15	Polar infall
12	20	20	19	(Probably a polar effect with no barometric change?)
13	24	24	24	Polar infall
14	29	29	29	Polar infall
15 May	2	2	2	Polar infall
16	8	8	9	Polar infall
17	15	15	16	Polar episode
18	19	19	18	Polar episode

It can be seen that the polar episodes on March 23, 25, 27, and 29 find their reflection in reticulocyte increase but with somewhat different summation in the two rabbits. It is also of interest to note that an unusually low count occurred around the ninth of April. With *pitressin injections* the total number of reticulocytes is greatly increased, though here, too, the rhythm of an association with meteorologic episodes is maintained for periods that are not involved in the pitressin injections as with crests 12, 14, 15, and 16.

In Fig. 3 we have the observations made on the same rabbits (1) and (2) during September and October of 1935. For the period preceding the injec-

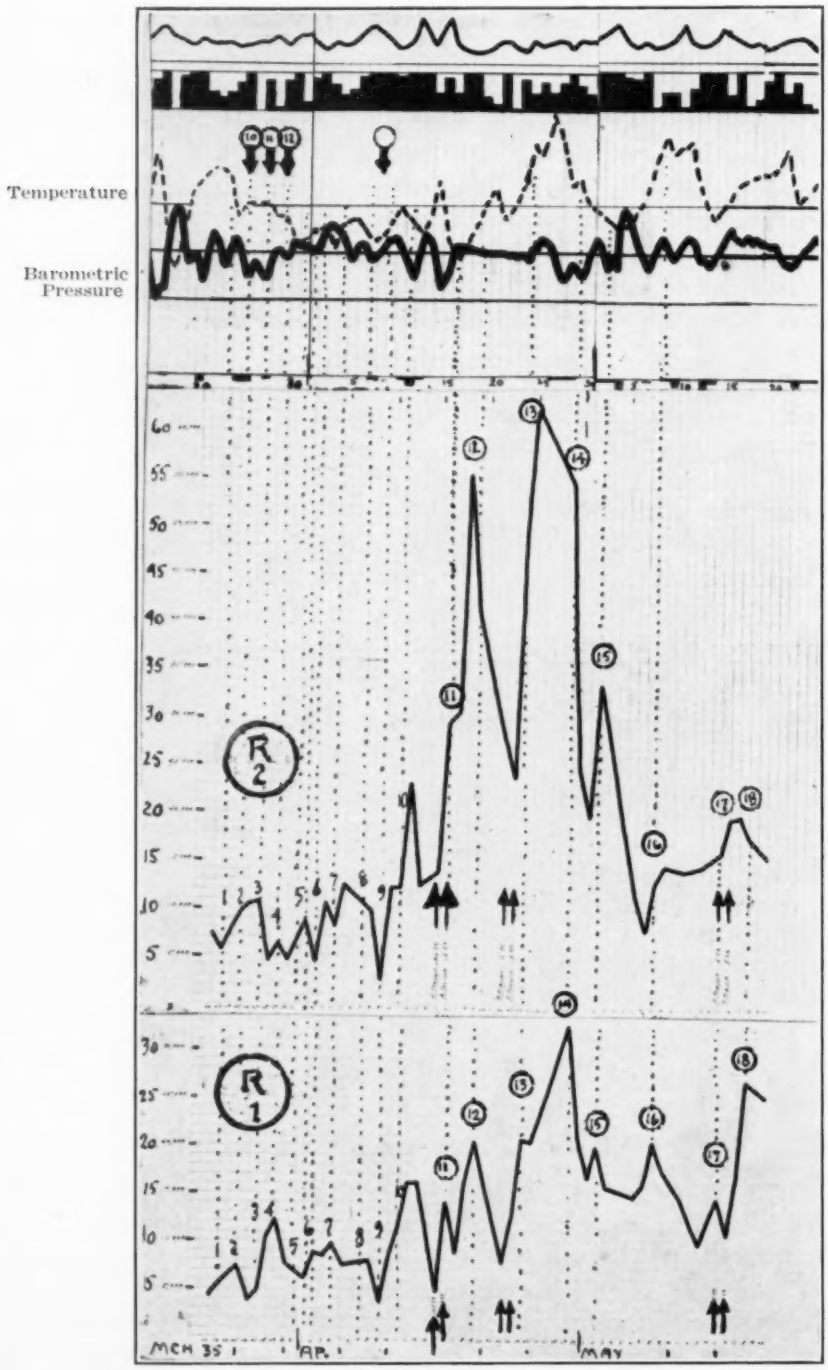

Fig. 2.—Basophilic aggregation counts in Rabbit 1 and Rabbit 2, with superimposed meteorograph for the time under consideration. Polar infalls have been numbered 1 to 18 and the corresponding reaction of increase in basophilic aggregates is indicated. Arrows pointing upward to days when pitressin was injected (0.5 c.c.).

tion of pitressin the days followed by an increase in reticulocyted count, are numbered 1 to 11, and for the period after pitressin, the actual days with reticulocyte crests are designated A, B, C, and D.

The synchronization is shown in Table II.

Here again we find that the reticulocyte peaks after the injection of pitressin exceed those occurring normally. These peaks are not as high as those pro-

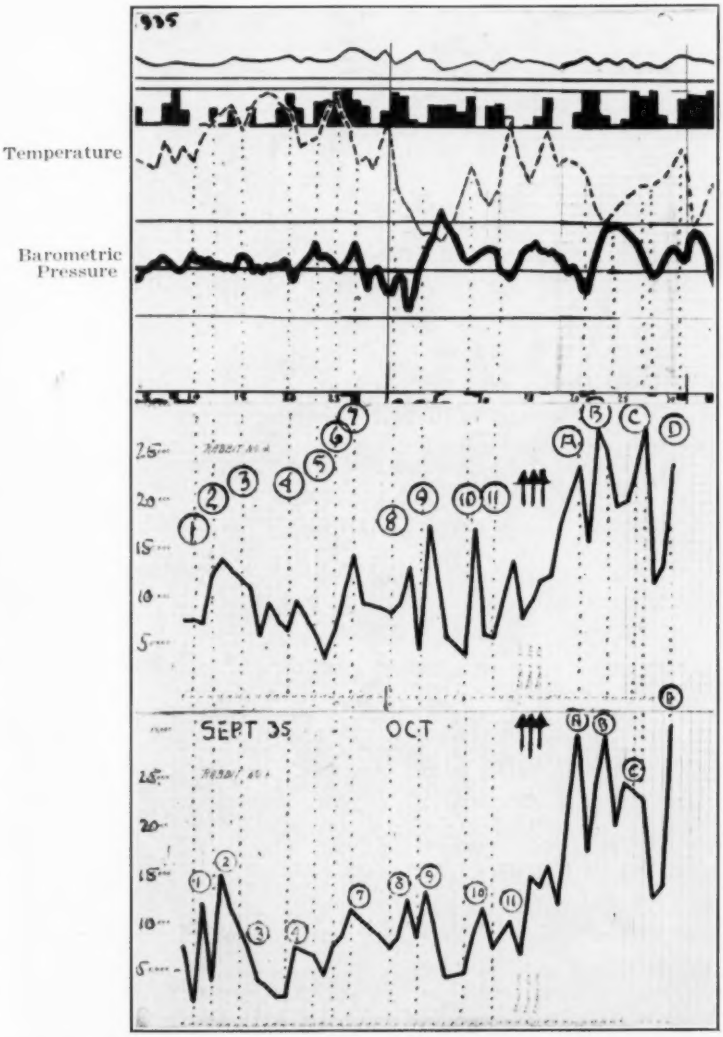

Fig. 3.—Basophilic aggregation counts in Rabbit 1 and Rabbit 2, with superimposed meteorograph for the time under consideration.

duced in the experimentation carried on during March and April, but this would be expected inasmuch as March and April are more disturbed meteorologically than September and October, and the summation of the natural and artificial pressor effects would be less during the less disturbed season. The normal rhythm is maintained in peaks A, B, C, and D inasmuch as each coincides with a naturally occurring polar episode.

TABLE II

METEOROLOGIC EPISODES	DATE OF EPISODES	DAYS FOLLOWED BY BASOPHILIC AGGREGATION PEAKS		METEOROLOGIC ASSOCIATION
		RABBIT 1	RABBIT 2	
1	September 10	11		Polar crest
2	12	12	13	Here the polar air masses passed to the north of Chicago but without marked barometric alteration
3	15	Crest is extended	16 Extended	Polar infall on eighteenth
4	20	21	21	Polar infall
5	23	23	-	Polar crest
6	25	26		Beginning polar infall
7	26-27	27	27	Polar crest
8	October 1	2-3	2-3	Polar infall
9	3-4	5	5	Polar infall (severe)
10	9	11	10	Beginning polar infall
11	12	14	14	Barometric crest
		After Pitressin		
A	21	21	21	Polar infall
B	24	24	23	Polar crest
C	27-28	26-27-28	28	Extension of polar episode
D	31	31	31	Beginning polar infall

CONCLUSIONS

Analysis of the data obtained, first of all reemphasizes what was observed previously, namely, that in basophilic aggregation counts a normal rhythm occurs, and that the rhythm is meteorologically conditioned.

After the injection of pitressin, the peaks attained were definitely higher than those occurring normally. This is in accord with the assumption that the normal peaks occur as the result of the biologic phase alteration with the anoxia of the ARS phase resulting in bone marrow stimulation. In pitressin we have employed a known pressor agent, and its use has resulted in a summation of natural and artificially produced pressor effects. That a pressor substance will affect the reticulocyte count has been reported by Dodds, Hills, Noble and Williams[3] who found a reticulocytosis as high as 50 per cent five days after the injection of B. P. pituitrin. That anoxia will result in reticulocytosis has been shown by Meyer, Seevers and Beatty[4] who report this finding in rats maintained for twenty hours at a barometric pressure of 282 mm. Our findings confirm the observation of Petersen[5] on the general integration of the environment and the functional status of the organism.

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ISOTRANSPLANTATION OF THYROID GLANDS IN DOGS*

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WE HAVE been interested in finding a method of successfully performing glandular homotransplants. The following report constitutes a study of thirty-six thyroid cross transplants in eighteen dogs. This study involved cross matching of the blood of dogs, basal metabolisms, postoperative clinical observation, and finally autopsy examination.

The experimental work of Halsted on auto- and isotransplantation of the thyroid and parathyroid glands is the greatest individual contribution on this subject. The successful transplantation by Payr in 1906 of a parathyroid glandule into the thyroid stimulated Halsted to investigate this subject completely. As a result of these studies he formulated the Law of Deficiency. This states that there must be more than a 50 per cent reduction in physiologic activity of a gland before the possibilities of a transplant can be assured. The only autotransplants that took were those in which a deficiency greater than one-half was created. Every isotransplant attempted by Halsted in spite of a created deficiency failed. The transplanted material was found completely absorbed or existing as a necrotic mass depending upon the time of inspection after the cross transplantation.

The mechanical factors in transplantation such as asepsis, the site of inoculation whether it be spleen, rectus sheath, or axilla, fragmentation of the gland, et cetera, have in no way contributed to the permanence of homotransplanted glandular tissue. Blood compatibility as a factor has been considered by previous workers, namely Lexer and Morris and Hess and Straus. The latter cross transfused their animals before homotransplantation of the thyroids was done. These transplants were all absorbed. Others (Underwood and Holman) believe that foreign protein sensitization is the important factor in absorption.

The work listed below represents eighteen total thyroidectomies and thirty-six single cross plants. The average weight of the dogs was about 13 kilos (28.6 pounds). The dogs were operated upon in pairs. The blood of each pair was cross matched and did not agglutinate. A basal metabolism was done in each case prior to operation. On the day of operation the animal received one gram of morphine sulphate followed in a half-hour by an intratracheal anesthesia of ether. Operating room technic and asepsis were rigidly observed in all cases.

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A cervical incision was made about 3 inches long. The neck muscles were separated in the midline and retracted laterally. The larynx was exposed and the thyroid gland visualized. The thyroid gland was removed complete after ligation of the blood supply at the upper and lower poles. Attempts in all cases were made to preserve the parathyroid glands in situ. The thyroid glands were placed in moist (Ringer's) warm laparotomy pads and were exchanged with the operator of the second dog. A midline abdominal incision was then quickly made. The peritoneum was opened and the omentum exposed. The capsule of the thyroid gland was fixed to the omentum by three black silk sutures. The omentum was then folded over to form a culdesac containing the gland. The pocket of folded-over omentum was closed by two or three sutures. The lower border of the omentum was always selected, and

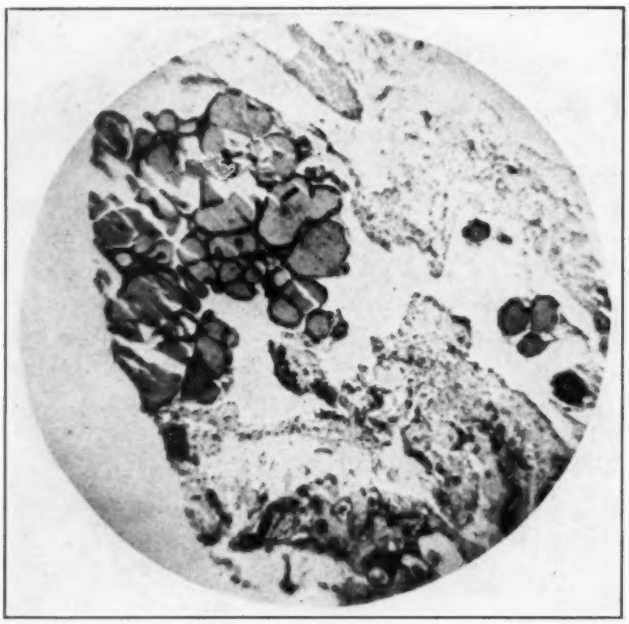

Fig. 1.—Showing thyroid gland in omentum cross planted eighteen days. The alveoli are distended with colloid which is deeply stained. There is no infection or involvement of the omentum.

its rich blood supply thus contacted all surfaces of the thyroid gland. Care was taken against strangulating any of the blood vessels by placing the sutures in avascular areas. A special abdominal incision was made and a pocket was formed between the fascia and rectus (9 cases) or between the rectus sheath and peritoneum (9 cases). The pocket was away from the incision and the second lobe of the thyroid gland was placed therein. Closure was made with black silk.

There were no immediate postoperative deaths. Four animals developed tetany of which three improved after treatment with parathormone and calcium chloride. All the animals received desiccated thyroid in their food the first postoperative week. Three animals developed superficial neck infections which cleared with surgical treatment. Only one animal developed an infec-

tion of the abdominal pocket. This thyroid was destroyed. Three animals died within two weeks of operation possibly due to acute thyroid deprivation. The first month after operation the animals were thin and lost much of their hair. They improved quickly, however, gaining in strength, weight, and hair growth.

Below we give some brief notes on the autopsy findings of dogs dead on the second postoperative day (tetany), the eleventh day (sacrificed), and the typical findings of those autopsied after three months.

Dog 24.—The neck incision was opened. A small amount of fluid showed in a clean operative field. The abdomen was opened through the operative incision. The omentum was found to be thickened at its distal portion. The vessels of the omentum were markedly

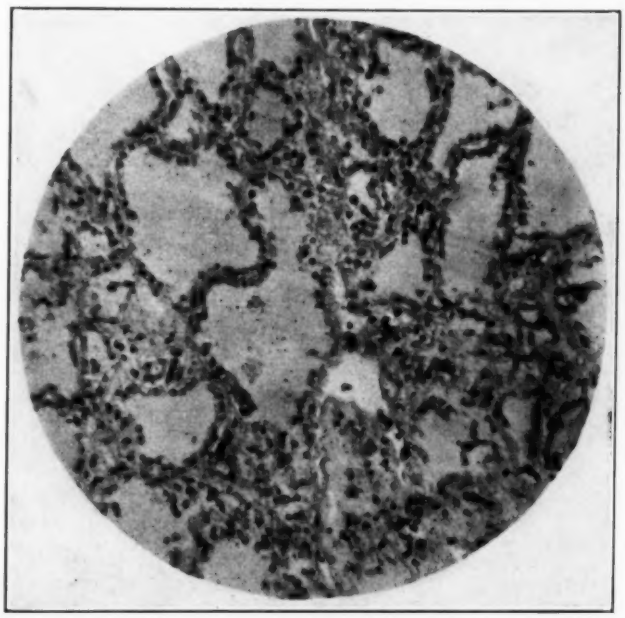

Fig. 2.—Thyroid removed after twenty-one days' implantation. Note the alveoli are distended. The colloid is practically gone with empty acini. There is a moderate amount of round cell infiltration.

injected. The thyroid appeared swollen and injected. The second thyroid under the fascia was hemorrhagic on its upper and lower surfaces. There was no sign grossly of peripheral or central necrosis in either gland.

Dog 4.—Eleventh postoperative day. Both thyroid glands were living. The thyroid gland in the omentum was surrounded by blood vessels. The omentum was thin and showed no signs of inflammation. There was no secondary infection or hematoma. The thyroid embedded within the posterior sheath had a definitely established blood supply.

Dogs 66, 33, 26, 22, 35, 62 when sacrificed after three months showed no traces of either the superficial or deep grafts. A deep brownish yellow stain of the tissue and remnants of black silk were the only indications of any previous surgery. The omentum appeared perfectly normal.

The basal metabolic graphs in our series were interesting. The animals showed no lowering of the basal metabolism. Early in this work we considered

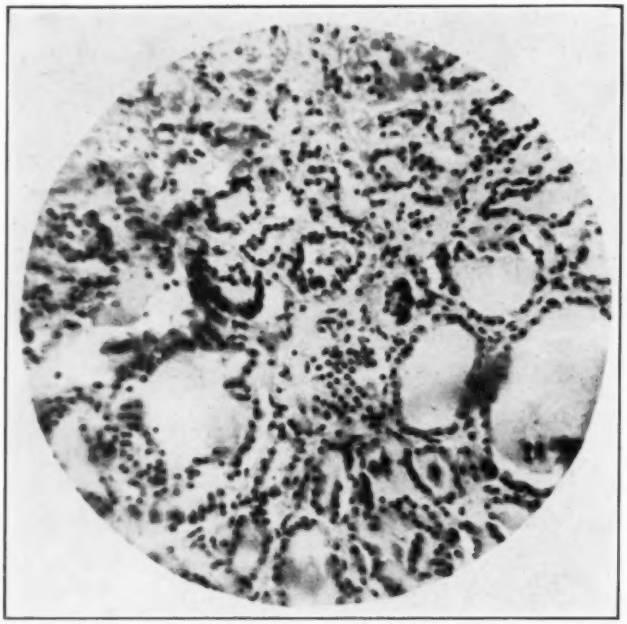

Fig. 3.—Section of thyroid gland after twenty-eight days' implantation in omentum, showing round cell infiltration of gland. Acini irregular and faintly staining.

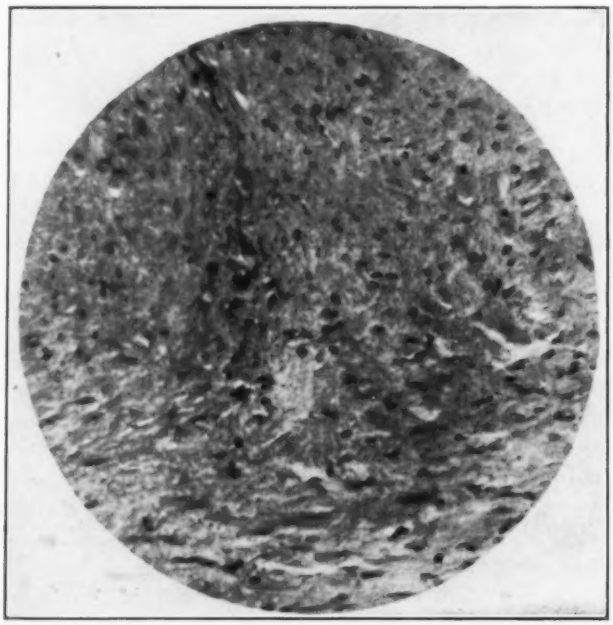

Fig. 4.—Tissue sectioned through area previously containing thyroid gland. There are practically no evidences of such implanation. Note remnant of black silk at the upper angle.

this as evidence of successful cross transplantation. This view we were forced to discard when the grafts were not demonstrable.

Autopsy examination revealed the presence of small bodies of accessory thyroid tissue located along the arch of the aorta or in the mediastinum of the dog. Halsted first recognized the existence of such accessory tissue but

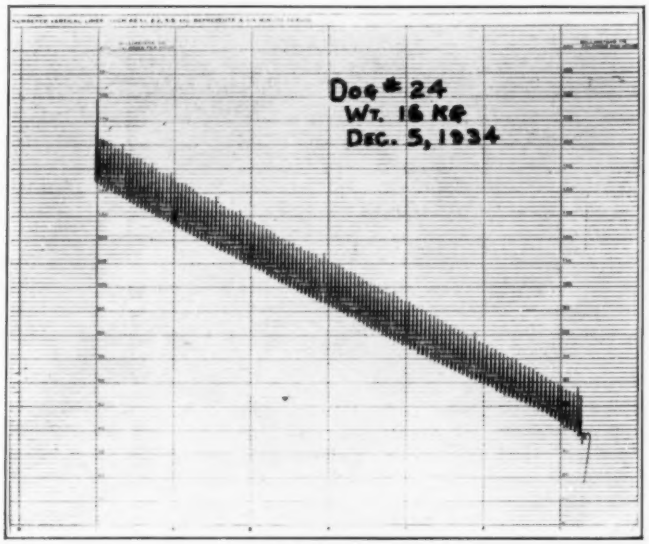

Fig. 5.

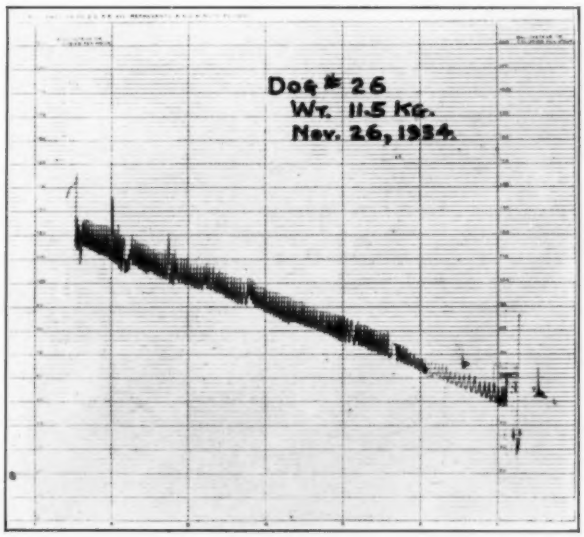

Fig. 6.

Figs. 5 and 6.—Basal Metabolic Graphs. Dog 26, three months after thyroid transplants. Dog 24, tenth postoperative day.

did not emphasize its importance. Swarts and Thompson found accessory tissue (thyroid) in 24 out of 30 dogs examined. We quote their findings: "Twenty-four of the animals had pericardial thyroid glandules. The total number of the glandules found was 68, as many as 7 being found in a single

animal. In 2 animals, 6 were found, 3 animals showed 5, 2 showed 3, and 12 showed 2. In 4 animals a single glandule was found." This accounts for the positive basal metabolism graphs we obtained. Though this accessory thyroid tissue appears minute in amount, it can sufficiently supply the body demands. We found no hypertrophy of these accessory glandules. Halsted emphasized this fact before.

SUMMARY

Thirty-six cross transplants were made in animals whose blood cross matched. After ninety days no evidences of living thyroid tissue could be found. Basal metabolic studies were unsuitable criteria of successful takes, as proved finally on autopsy examination. The existence of accessory thyroid tissue no matter how grossly small was sufficient in dogs to fill body requirements without addition of foreign gland grafts.

The authors gratefully acknowledge their indebtedness to Professor Arthur M. Wright and Dr. CoTui.

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THE TREATMENT OF RHEUMATOID ARTHRITIS WITH FORMALIZED STREPTOCOCCUS FILTRATE (TOXOID)*

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THE subject of arthritis needs no introduction, as the literature on this subject has been abundant during the past decade, due to the interest which has been stimulated in the study of arthritis by the International League for the Study and Control of Rheumatic Diseases.

It is not the purpose of this paper to review the literature of the subject. A complete and detailed résumé of the American and English literature on "Rheumatism" and arthritis was recently published by Hench, Bauer, Fletcher, Ghrist, Hall and White,[1] and the reader is referred to this review for classification, etiology, pathology, laboratory procedures, theoretical considerations, and various methods of therapy, as well as the names of all the workers in this field and their contributions.

TREATMENT

The difficulties in the treatment of rheumatoid arthritis are generally admitted not only because of the different degrees of involvement and stages of the disease in different patients, but also because of the variety of treatments and remedies offered and used, among which are included: (1) Many drugs, chemicals, and various compounds, including endocrine preparations; (2) diet; (3) vitamins; (4) climate; (5) physiotherapy, including ozone, hot air conditioned cabinets, and colonic irrigations; (6) artificial fever; (7) surgery (synovectomy, arthroplasty, arthrodesis and sympathectomy). Injections used include: (8) Many varieties of arsenicals; (9) gold salts; (10) silver salts; (11) iodized oil; (12) colloidal sulphur; (13) histamine; (14) choline salts. (The latter two with or without the galvanic current.) (15) Many varieties of milk preparations; (16) foreign proteins of all types; (17) typhoid vaccine; (18) other vaccines, in single or multiple forms.

VACCINE THERAPY

Vaccine treatment with the streptococcus, which has been stressed by many ardent workers and has occupied much space in the literature of recent years, has at best shown but limited success. In general, cases in earlier stages improved much more frequently than those in later stages; in the latter group vaccine treatment did not prove very encouraging.

It was because of the limited success and the unconvincing rationale of therapy that it was decided to broach the subject from a different angle. According to the investigations of Rich[2] and others it has been fairly well estab-

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lished that certain infections and reinfections, and foci of infection, tend to sensitize certain, if not all, individuals to the bacteria and their products of metabolism, to which products these individuals become more or less allergic, (bacterial hypersensitiveness). This allergic manifestation which is present in chronic infection can be readily shown by intradermal testing with the products or soluble toxins of these bacteria. Active clinical disease in a chronic form will occur in such allergic individuals, and a rheumatoid arthritis will develop in a patient who has such a hereditary tendency and constitutional predisposition (arthrotropic). It may also be said that chronic streptococcic disease is a definite clinical entity, as much as chronic tuberculous disease, and the allergic mechanism is probably similar.

Fig. 1.—Intradermal reactions. *A*, Streptococcus toxin, immediate reaction. *B*, Sterile broth control, immediate reaction. *C*, Streptococcus toxin, twenty-four-hour reaction. *D*, Sterile broth control, twenty-four-hour reaction. Note that the immediate reactions are alike in both the toxin and sterile broth control.

It was thought, therefore, that if these patients could be treated with the same substances to which they are allergic, beginning with a small dose and gradually raising the dosage in accordance with the patient's reaction or response, there might result an antigenic stimulation of some value. However, the danger of injecting toxin broth filtrate was carefully considered, as it might throw an additional hazard upon subvitalized tissues. It was, therefore, reasoned that if these toxins were detoxified or modified, it might prove an agent of low toxicity and high antigenic value to stimulate a response in these depreciated tissues. This was attempted as follows: Strains of streptococci were isolated from patients with active rheumatoid arthritis. Cultures were

made from roots of teeth, tonsils, throat, nasopharynx, nose, sinus puncture, urine, and stool. These were grown in beef heart broth, with a pH of 7.7 to 7.8. Only the strains having potent soluble toxins were used. The filtrates were then treated with formalin, the same as in diphtheria toxoid. Dilutions were then made of 1:10, 1:100, 1:1,000 and 1:10,000. Treatment by subcutaneous injection was begun with 0.1 c.c. of the highest dilution and increased gradually depending upon the patient's reaction to the original test dose of the toxin and the patient's reaction to the therapeutic dose. No rule of thumb

Fig. 2.—Delayed reactions, twenty-four-hours. *A*, Hemolytic streptococcus toxin. *B*, Boiled toxin, the same as *A*. *C*, Nonhemolytic streptococcus toxin. *D*, Tuberculin 1:100, Mantoux.

or standardized method of treatment has been developed so far. *Individual dosage is essential and must be worked out for each patient.* A very slight local reaction at the site of injection was considered favorable, and was used as a guide in raising the dosage. The intradermal tests were used as indicators of the type of streptococcus probably responsible and the one giving the strongest reaction was used in treatment, but only in single strain. Whenever possible autogenous material was used. Otherwise treatment was given

with the stock strain giving the strongest reaction. Treatment was given twice a week and continued for at least three to six months, and in many cases much longer. Occasionally a patient reached 0.1 c.c. of a 1:10 dilution and could take no higher dosage, except at the risk of a marked local reaction lasting many days, and at times a focal or constitutional reaction. The latter reactions are uncomfortable, *never dangerous,* but are to be avoided, as they tend to bring the patient into a higher allergic state and interfere with the attempted desensitization.

At first treatment was begun very guardedly in patients with advanced stages of the disease, of many years' duration, and with marked deformities. The clinical results, subjective and objective, were not only encouraging but astounding, even though not manifest at times, before some months had elapsed.

Fig. 3.—Intradermal reactions. *A*, Tuberculin reaction (Mantoux) forty-eight hours. *B*, Streptococcus toxin reaction twenty-four hours.

There were objective signs of definitive improvement, locally and constitutionally. Subsequently, treatment was begun on a larger scale, and included many types of patients in various stages of the disease.

SOME ILLUSTRATIVE CASES

CASE 1.—R. Q., white, female, twenty-three years old, bookkeeper, was admitted to the Medical Service of the Kings County Hospital, on Oct. 27, 1932, with the diagnosis of infectious polyarthritis. About six to eight weeks prior to the onset of the arthritis the patient had had a severe grip and had been confined to bed for two weeks. The arthritis was acute, and involved all the joints of the right upper and left lower extremities. The temperature ranged from 101° to 103° F. for two months, and the patient was going downhill rapidly with marked emaciation and excruciating pains in the joints, requiring narcotics frequently. The heart findings were interpreted as due to rheumatic fever, while the changes in the joints as

those of rheumatoid arthritis. The prognosis was given as grave. At about this stage in her clinical course, at the end of December, 1932, treatment with modified streptococcic toxin was begun and given twice weekly. After six weeks of treatment, the patient began to improve gradually, moved the joints slightly without pain, began to take nourishment better, and an increase in strength was noted. Four months after treatment was begun, the patient was out of bed on crutches and a month later she was discharged from the hospital in excellent condition without any pain in any of her joints, and walking well. She gained so much weight that the general impression was that she doubled her weight in five months. The temperature came down to normal one month after treatment was begun and remained normal until she was discharged.

CASE 2.—L. E., white, female, twenty-eight years old, married, was admitted to the Brooklyn Jewish Hospital on March 25, 1933, with a diagnosis of infectious polyarthritis and ulcerative colitis (18 to 20 stools in twenty-four hours). The illness was of six months' duration and began with the colitis, followed a few weeks later by the arthritis. A blood study showed a marked anemia, hemoglobin, 45 per cent; red blood cells, 1,800,000; white blood cells, 16,500 with 68 per cent polymorphonuclears; sedimentation time, twelve minutes (Linzenmeier). The urine examination was negative except for a trace of albumin; complement fixation test for gonococcus was negative. The temperature ranged between 99° and 103° F. daily, and she was gradually getting worse with marked emaciation, anorexia and increasing anemia. She received two transfusions, each consisting of 500 c.c. of whole blood without any effect on her downward course. When first seen by me, she had been at the hospital for six weeks, her status and clinical course being as described. The condition of the joints was as follows: all the joints were painful and tender, the joints of the fingers, wrists, elbows, knees, ankles, and feet were swollen, hot, stiff, and spindle-shaped, with considerable muscular atrophy. There were beginning deformities of the hands, wrists, elbows, and knees, the latter being contracted and forming an angle of about 90 degrees with the thighs. X-ray showed no destruction of joints, and the articular surfaces were intact but the spaces between them were narrowed. A study of the stool was made, and after eliminating the *B. coli* in a carbonate solution, a toxic strain hemolytic streptococcus was isolated. A toxoid was prepared, and in the meantime treatment was begun with a stock toxoid of a similar streptococcic strain, injections being given every other day, beginning with 0.1 c.c. of 1:100,000 dilution. The pain and swelling of all the joints began to diminish after the first six injections. The contractures were gradually released, and the patient regained all joint function after six weeks of treatment, which was intercepted with the autogenous toxoid as soon as it was ready. She was out of bed and began walking fairly well. Her ulcerative colitis improved at the same time to the extent that the number of stools were reduced from 18 or 20 to one or two a day, well formed. She gained considerable weight and was on a full diet when discharged from the hospital, July 11, 1933. This patient showed the most rapid change and marked improvement under this form of therapy. No other measures were used while this treatment was given, neither for the colitis nor the arthritis.

CASE 3.—N. B., white, female child, eleven years old, was admitted to the Pediatric Service of the Kings County Hospital on May 2, 1933, with a diagnosis of infectious polyarthritis. Two months previously she had had an otitis media, and several weeks following this infection she began to have pains in the elbows and hips and soon after the other joints became involved. At the time of admission to the hospital, the right elbow was already ankylosed at about an angle of 90 degrees, the patient was on her back with the knees apparently ankylosed, forming an angle of contracture with the thighs of about 60 degrees. She was extremely emaciated, and all her joints were painful and stiff. She was transferred to the neurologic service as a case of poliomyelitis, but was sent back to pediatrics as a case of tuberculosis, and was then referred to orthopedics because of the marked deformities. Opinions differed from poliomyelitis to tuberculosis, to Still's disease. X-ray films showed changes in the hips, knees, and elbows which were interpreted as due to an infectious process. Mantoux (old tuberculin 1:50) was negative. Blood Wassermann was negative. Repeated vaginal smears and complement fixation were negative for gonococcus. There was a moderate anemia of 50 per cent hemoglobin, 2,680,000 red blood cells, and a leucocytosis of 14,000 with 70

per cent polymorphonuclears. The temperature ranged between 98.6° F. and 100.5° F., and the pulse was moderately elevated. Treatment with modified streptococcic toxin was begun in June, 1933, and continued for ten months on the Orthopedic Service of Dr. J. L'Episcopo, which service gave every conceivable help in the rehabilitation of the child. A note made by the Orthopedic Service on the chart on Dec. 12, 1933, states: "knees and ankles freely movable, no pain anywhere, right elbow fixed." The patient was discharged May 2, 1934. She had gained considerable weight, was pain free, and was able to walk without help. This patient was last seen on Oct. 10, 1935. She looks well and feels well, has had no recurrence of pain or any other joint symptoms during the past eighteen months, without any therapy. If it were not for the ankylosis of the right elbow with which she was first admitted to the hospital, it would be difficult to tell that the child had been so severely ill with a deformative arthritis.

CASE 4.—M. M., white, female, fifty years of age, houseworker, was admitted to the Medical Service of the Kings County Hospital on Aug. 9, 1932, with the complaint of pain and swelling of all the joints of the upper and lower extremities of several weeks' duration. She ran a slight fever of 99° to 101° F. for a month. She had had a similar attack three years previously and was bedridden for eight weeks. The present attack was much more severe and the patient continued to get worse with marked emaciation and beginning deformities of hands, wrists, elbows, knees, and ankles, and periarticular muscular atrophy. She was considered a permanent invalid. Consequently she was depressed mentally. A urine examination was negative. Her blood pressure was low, 100 systolic and 65 diastolic. Blood Wassermann was negative and a blood study showed a moderate anemia. Treatment with modified streptococcic toxin was begun in December, 1932, four months after her admission to the hospital, and continued twice weekly for a month and then once a week for four months. She was discharged from the hospital on July 27, 1933, in excellent condition, with perfect use of all her joints, freedom from pain, and with considerable gain in weight. After a rest in a convalescent home for several weeks, she went back to her job, doing housework.

TABLE I

SUMMARY OF TREATMENT OF 100 CASES OF RHEUMATOID ARTHRITIS

Number of cases	100			
Males	25			
Females	75			
Marked improvement	43	} 69	(favorable results)	
Moderate improvement	26			
Slight improvement	16	} 31	(failures)	
No improvement	15			
Males	25			
Marked improvement	14	56%	} 80%	
Moderate improvement	6	24%		
Slight improvement	3	12%	} 20%	(failures)
No improvement	2	8%		
Females	75			
Marked improvement	29	39%	} 66%	
Moderate improvement	20	27%		
Slight improvement	13	17%	} 34%	(failures)
No improvement	13	17%		

Marked Improvement: Disappearance of pain, swelling greatly reduced or disappeared, function greatly increased or returned to normal.

Moderate Improvement: Pain greatly diminished or disappeared, but recurs occasionally. Swelling receded, but not completely and permanently. Function improved. Occasional transient exacerbation.

Slight Improvement: Pain and swelling occasionally disappeared but recurs frequently, especially in change of weather.

CASE 5.—E. G., white, male, twenty-eight years old, was first seen on the Orthopedic Service of Dr. L'Episcopo at the Kings County Hospital in July, 1933. The history was as follows: In 1925, he began to complain of pains in the joints of his feet and was treated at the New York Mount Sinai Hospital, where at that early stage Dr. Emanuel Libman gave a bad prognosis. A tonsillectomy was done and he felt improved for about a year during which time he passed the examination and joined the U. S. Army. In 1926, he again developed pains in the feet, and also in the knees and hips. He was admitted to the Walter Reed Hospital and was kept there for twelve months. In November, 1927, he was readmitted to the Walter Reed Hospital on the complaint of pain in the wrists. This was a mild attack and he improved and was discharged after several weeks. In 1930 he was readmitted to the Walter Reed Hospital and this time his entire spine was involved in addition to the joints of the extremities. He was at the hospital for thirteen months and the spine was fixed. In 1932, he was admitted to the Soldiers' Home Hospital as a chronic invalid, and was to stay there indefinitely. In July, 1933, his uncle, a Brooklyn physician, wrote to him and following the communication, the patient asked to be discharged from the Soldiers' Home Hospital, came to Brooklyn, and was admitted to the Kings County Hospital for observation and study. Treatment with modified streptococcic toxin was begun at the hospital in July, 1933, and was continued after he was discharged from the hospital four months later, at first twice weekly and after six months, once a week. After ten months of treatment he made remarkable progress, and could move his neck, shoulders, hips, and knees much better, and had great relief from pain and stiffness. Occasionally he gets some pains in the back of the neck and hips on change of weather, but considering the obvious limitation of therapy in such a patient, his improvement is considered very satisfactory. He is still under observation and treatment and is seen at fairly regular intervals. His mental state of depression for many years, during which time he frequently talked of suicide, changed completely. He made good adjustment, and expressed a desire to study engineering.

CASE 6.—M. D., white, female, thirty-seven years old, married, was referred to me on Sept. 19, 1933, by the late Dr. W. Bruce Anderson, with the diagnosis of advanced rheumatoid arthritis with marked deformities and muscular atrophy of seven years' duration. The history in brief was as follows: Pain in the joints of the feet began seven years ago, kept on with improvement and recurrences for several years and then the joints of the hands became involved. A tonsillectomy was done at this time, and she felt improved for about six months. During this period of improvement she had her first baby, and several months following the delivery, the pains recurred in the hands and feet. Two years after the first delivery she gave birth to a second baby, and following the second delivery, all the joints of the upper and lower extremities became involved. She was bedridden most of the time, and at best could sit in a wheel chair, frequently being unable to feed herself. When she was first seen by me, the hands were deformed with marked atrophy of the interossei muscles, the wrists were very swollen and stiff, the right elbow was contracted at about an angle of 90 degrees, the shoulders and hips were immobile, the knees were very swollen and partially contracted and the ankles and feet were similarly swollen, the left ankle being about twice the size of normal. She could not walk, and could barely stand up, but not without support. Her blood pressure was 140 systolic and 90 diastolic, red blood cell study showed a moderate anemia, white blood cells were 6,200 with 76 per cent polymorphonuclears. Urine examination was negative, blood chemistry normal, blood Wassermann and Kahn tests and complement fixation for gonococcus were negative. Her sedimentation time (Linzenmeier) was eighteen minutes. A complete bacteriologic survey of possible foci of infection was undertaken, and a nonhemolytic streptococcus was isolated from her nasopharynx and a similar organism from the stool. Intradermal tests with these organisms were strongly positive and lasted for a week. An autogenous toxoid was prepared and treatments were given twice weekly. After four months of treatment the patient was free from pain, the joints were remarkably improved; she was able to walk and to travel on the street car to the office, and two months later, i.e. six months after treatment was begun, she was able to do her own housework and take care of her family. She still returns regularly about once a month for observation, and she feels entirely well.

TABLE II

AGE INCIDENCE AND RESULTS THEREIN

AGE IN DECADES	MARKED IMPROVEMENT	MODERATE IMPROVEMENT	SLIGHT IMPROVEMENT	NO IMPROVEMENT	TOTAL PER DECADE
10		1			1
20	3	1			4
30	5	5		1	11
40	16	7	6	4	33
50	10	5	4	4	23
60	8	5	5	5	23
70	1	2	1		4
80				1	1
Total in each group	43	26	16	15	100

COMMENT

An analysis of Table II shows the age incidence in decades, represented by the last vertical column, and the results of treatment in each decade, shown by each horizontal column, separated into the respective groups. For instance, in the fifth decade, the total number treated is 23. This number is found in the last vertical column, on the horizontal column between 40 and 50 (see Table II). Of these 23 patients, 10 were markedly improved, 5 moderately improved, 4 slightly improved, and in 4 there was no improvement at all. The same analysis is made of the groups in other decades. The bottom horizontal column gives the total number in each group of results, including all ages. The last number in this column gives the total of all these groups as well as the total of all decades in the last vertical column.

SUMMARY

An analysis of 100 cases of rheumatoid arthritis is herewith presented. Treatment with formalized bacterial filtrates was based on the premise that chronic nonspecific infectious arthritis presents a problem of reinfection of a previously sensitized host akin to the mechanism now accepted in tuberculosis. The results justify this method of approach and warrant its continuation. Whether the procedure involved represents the usual nonspecific protein treatment or the so-called anamnestic reaction is difficult to tell at present. Continued observations and studies of these patients from different angles as to the effect of treatment and the body responses caused by this effect may throw further light on the value of this therapy.

I wish to acknowledge my indebtedness to Dr. B. Koven, who is responsible for stimulating the initiation of this study and who was generous in the supply of material from his Orthopedic Service at the Jewish Hospital. Also, I wish to express my gratitude to Dr. J. L'Episcopo, Chief Orthopedic Surgeon of the Kings County Hospital, who was keenly interested in the problem and who permitted the use of the material in his orthopedic wards for this study.

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61 EASTERN PARKWAY

BLOOD CHEMISTRY IN HOOKWORM ANEMIA*

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VERY few publications devoted to the study of blood chemistry in hookworm anemia have appeared in the literature. Protein determinations in two cases by Vadala[1] in Italy and fatty acids and total lipids by Oswaldo de Oliveira[2] and J. Villela[1] in Brazil, are the only known papers dealing with this question. It can be said that most of the work done in hookworm disease is dedicated to hematologic studies. The chemical aspects, with the exception of hemoglobin, have not yet been considered. However, clinical studies have called attention to some disturbances like edema and undernutrition with which chemical changes should be correlated.

In 1929 we published our first report on the blood chemistry of hookworm anemia.[3] Calcium, potassium chlorides, cholesterol and alkali reserve were then studied. Calcium determinations showed normal values (8.0 to 10.8 mg. per 100 c.c.) and potassium slight lower average (16.3 to 20.30 mg. per 100 c.c.) than normal, probably caused by the decreased number of red corpuscles. Cholesterol was only investigated in a few cases and nearly normal figures were found, Bloor-Sackett's technic being employed.

The most interesting fact then observed dealt with the marked fall in the total serum protein. In a subsequent paper[4] (1930) this question was reinvestigated and Howe's technic was adopted for the determination of the albumin and globulin fractions and a gravimetric one for fibrinogen. These analyses showed that the fall in proteins previously observed was due to a decrease in the albumin fraction, the globulin being unaffected or slightly increased. This fact was afterward confirmed by F. Ribeiro[5] in Brazil (1931) and by Eerkens[6] (1932) in Java.

In the present paper the chemical changes in blood plasma of patients with hookworm anemia are shown, and a suggested correlation between these changes and edema production are given.

Normal values for plasma proteins varied from 7.0 to 8.8 gm. per 100 c.c. (Rowe, Hammarsten, Patein, Myers, Epstein, Salvesen, Codounis, Wiener and Wiener[7-9]). Our normal values obtained in preabsorptive conditions have shown an average of 7.82 gm. (7.0 to 7.9). Howe's technic was employed in all cases for protein fractions and Chandler's was employed for fibrinogen.[10, 11] Linder, Lundsgaard and Van Slyke[12, 13] reported 3.4 gm. for the albumin and 2.3 to 2.9 for the globulin fraction of blood plasma. Our findings varied from 4.4 to 6.0 gm. (average 5.1) for the albumin and 1.8 to 2.8 gm. (average 2.4 gm.) for the globulin.

*From the Oswaldo Cruz Institute.
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The 14 patients studied presented different aspects of the disease. All of them presented marked anemia and 9 showed edema. Protein and cholesterol determinations were made in blood plasma. In 5 cases the blood was analyzed periodically for its protein content before and after treatment.

Inspection of Table I reveals a marked decrease in the amount of protein in all cases presenting edema. Lower values are found when edema is more accentuated. However, temporary high values may be observed during periods of dehydration. Old cases present lower protein values than recent ones. Acute infestation seems to be of greater influence than a chronic one in lowering plasma proteins.

TABLE I

PLASMA PROTEINS IN PATIENTS WITH HOOKWORM ANEMIA

INTENSITY OF EDEMA	VALUES IN GR. PER 100 C.C. OF PLASMA				HEMOGLOBIN (SAHLI) %	ERYTHROCYTES MILLION PER 1 MM²
	TOTAL PROTEINS	ALBUMIN	GLOBULIN	FIBRINOGEN		
++	6.7	3.7	3.0	–	55	3.1
+	7.1	5.0	2.1	0.30	32	2.8
++	6.4	4.0	2.4	–	31	2.7
+++	6.5	3.7	2.8	0.48	28	2.0
+	6.6	3.8	2.8	0.47	39	1.9
+++	5.6	2.5	3.1	0.60	25	2.2
+	6.8	4.7	2.1	–	70	3.5
++	5.8	2.8	3.0	0.56	21	2.0
++	5.3	3.1	2.2	–	20	1.2
0	7.5	5.3	2.2	–	66	3.8
0	7.4	4.9	2.5	–	67	3.1
0	7.1	4.0	3.1	–	48	2.1
0	7.1	4.2	2.9	–	61	3.0
0	7.2	4.5	2.7	–	32	1.8

Average for total proteins { With edema 6.3 / Without edema 7.2

Average for albumin { With edema 3.7 / Without edema 4.5

Treatment with antihelminthic drugs associated with ferrous salts and good diet raises plasma protein with a parallel reabsorption of edema. In the absorption period some variations are observed which decrease or maintain stationary the amount of plasma proteins (Chart 1). Only after one or two months can normal values be found.

One of the most important functions of the plasma proteins is the maintenance of the physicochemical state of the blood. The fluid balance between the blood and the intercellular tissue spaces and serous cavities results on the one hand from the osmotic attraction for water, and, on the other, from the hydrostatic pressure of capillaries.

Starling, in classical experiments, demonstrated this fact by measuring by dialysis the osmotic pressure of plasma proteins. Govaerts[15] and Schade and Claussen[14] developed more accurate technics and confirmed Starling's conclusions. Govaerts[15] studying separately globulin, albumin, and fibrinogen in normal human plasmas observed that albumin exerts an osmotic pressure of 7.54 cm. of water, and globulin 1.95 only. The plasma proteins, chiefly albu-

min, are in opposition to hydrostatic pressure of capillaries, edema appearing when the colloidosmotic pressure of protein (or "oncotic pressure" of Schade and Claussen) falls below a given limit.

The clinical and physiologic studies of Epstein, Leiter, Darrow and Hopper confirmed brilliantly the theory proposed first by Starling and developed afterward by Govaerts.

We suppose in our cases of hookworm anemia that edema appears consequently to a low oncotic pressure due to the marked decrease in the albumin fraction. The calculation by means of Govaerts' factors gives us low figures for the colloidosmotic pressure. A correlation between the latter and the presence of edema is shown in Table II.

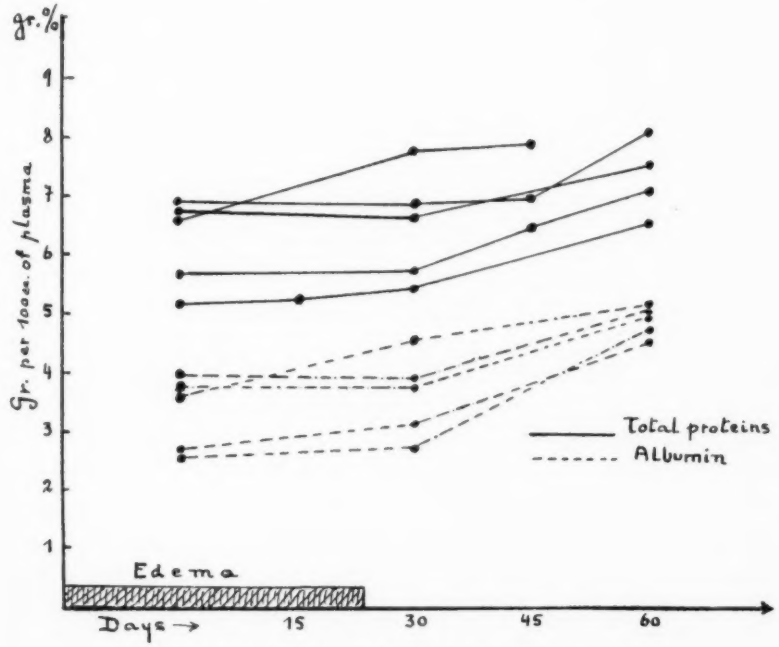

Chart 1.—Plasma proteins during treatment.

In malnutritional and cachetic edema low figures for plasma proteins and the relation between the nature of edema and hypoproteinemia are frequently shown as reported by many authors (Knack and Neumann, Kohman, Maver, Fritsch, Mendel and Peters, Bruckman, D'Esopo and Peters[13, 16]).

In hookworm anemia a hyponutritional state is commonly found and edema can be attributed to the decrease in plasma proteins. According to Moschcowitz[17] three different kinds of disorders may be responsible for hypoproteinemia: loss of proteins (by kidney, digestive tube, hemorrhages), insufficient production and diet poor in proteins. In nephrosis and nephritis the wastage of proteins is due to their loss in urine. On the other hand, in hookworm disease albuminuria is slight and the decrease in plasma proteins cannot be attributed to this cause.

TABLE II

EDEMA, COLLOIDOSMOTIC PRESSURE OF PLASMA PROTEINS AND CHOLESTEROL

INTENSITY OF EDEMA	COLLOIDOSMOTIC PRESSURE IN CM. OF WATER (CALCULATED BY GOVAERTS' FACTOR)		TOTAL PROTEINS IN GR. PER 100 C.C. OF PLASMA	CHOLESTEROL IN MG. PER 100 C.C. OF PLASMA
++	28.4		6.7	-
+	41.7		7.1	-
++	34.8		6.4	-
+++	33.3		6.5	139
+	34.1		6.6	122
+++	24.8		5.6	122
+	38.5		6.8	152
++	26.9		5.8	94
+	27.6		5.3	120
0	44.2		7.5	133
0	41.8		7.4	142
0	36.2		7.1	103
0	36.3		7.1	151
0	39.1		7.2	86
Average	With edema	32.2	6.3	124
	Without edema	39.5	7.2	123

In our opinion the most important factor that affects the albumin content of plasma in hookworm disease is the state of undernutrition found in the majority of cases associated with a constant loss of blood by way of the intestinal canal. Those patients without edema and therefore with normal values for plasma proteins are well nourished, although there may be intensive intestinal infestation by parasites. When proteins are added in large proportion to the diet (beef), edema disappears and plasma proteins rise to normal level, even without antihelminthic treatment. Iron introduced by high meat diet in some cases contributes to the reabsorption of edema and in this way the patients recover a normal blood picture, according to the studies of W. Cruz.[18]

Bruckman and Peters[16] have shown that edema appears in patients with malnutrition when serum albumin is below the normal level. Wastage of serum proteins is probably the most important feature of such malnutrition. In protein-starved rats edema has been observed by Kohman, Fritsch, Mendel and Peters and more recently (in low protein diet) by Torbert.[19]

Edema was frequently observed during the World War due to the lack of proteins in diet and low figures in blood plasma were found (Schittenhelm and Schlichts). In beriberi cases one of us[20] found low plasma proteins in those patients showing edema. With recovery the edema disappears and the plasma proteins increase to normal level.

Chart 1 shows that proteinemia is also influenced in hookworm anemia by treatment and is related to the presence of edema. In two cases a slight fall in proteins was observed, probably due to the resorption of edema fluids.

The determination of cholesterol in plasma (Bloor and Knudson's technic) shows values situated toward the lower limit admitted for normal standard. In Brazil the normal average established by Villela and Silva[21] was 172 mg. for both sexes. The average found in 11 cases of hookworm anemia was 124 mg. per 100 c.c. of plasma. In all cases treated the cholesterol content in-

creased and attained normal levels. The hypocholesterolemia observed in this disease seems to be related to a diet poor in cholesterol. In well-nourished cases higher values are found.

Man and Gildea (in 10 malnourished patients who were followed for some time) found that the cholesterol of plasma varied with the state of nutrition. Values below normal were found in the majority of cases studied and are usually present together with hypoproteinemia.[22]

The kidney injuries observed in hookworm disease and belonging to a fatty nephrosis (Ashford and Igavaniesh) are not of the same kind as in lipoid nephrosis and have no influence on the renal function. Hypocholesterolemia and hypoproteinemia are produced by a malnutritional state and are not related to renal disorder.

CONCLUSIONS

1. In hookworm anemia with edema low values for plasma proteins are frequently found.

2. Hypoproteinemia is due to the decrease in the albumin fraction only, the globulin being unaffected or slightly increased.

3. Our observations suggest that the decrease in plasma albumin must be due to a lack of protein in the diet and to the state of undernutrition frequently found.

4. Edema in hookworm disease seems to be caused by the decrease in the colloidosmotic pressure of blood proteins consequent to the fall in its albumin content. It seems to be produced by a mechanism similar to the edema observed in cases of malnutrition.

5. Low values for cholesterolemia were found and are attributed to a diet poor in cholesterol.

6. Treatment associated with rich protein diet raises the protein content of plasma and therefore contributes to the disappearance of edema.

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SNAKE VENOM (MOCCASIN) IN THE TREATMENT OF EPILEPSY*

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STORIES of the alleged freedom from seizures of epileptics after having been bitten by snakes of the adder family were followed by enthusiastic reports by Spangler of snake venom (crotalin) treatment in epilepsy.[1-4] Spangler[3] said that not only are the virulence and number of epileptic fits favorably influenced by crotalin, but the excitability of the nervous system is modified and the general health of the patients, their mental faculties and metabolism, in every respect, are considerably improved. Anderson[5] reported the death of a patient under crotalin treatment and strongly urged against this therapy on the basis of the bacterial contamination of the venom then in use, and its antibactericidal action. Spangler[4] thought that crotalin solution was favorable in epilepsy because of its power to decrease the rate of coagulation of the blood, which he thought was increased in epilepsy. Thom[6] reported unfavorable effects of crotalin treatment and opposed the foundation upon which the treatment was based. He found the coagulation rate of the blood of epileptics to be within normal limits.

Of Thom's 14 cases of epilepsy on crotalin treatment, one developed hysterical attacks, in 6 the severity and frequency of convulsions increased, one, a case of hystero-epilepsy showed improvement, one had a violent local and systemic reaction, and there was no change in the rest. In 1926 Fackenheim[7] reported favorable results following venom treatment in epilepsy. He had been treating his patients since 1911[8] following the reports by Spangler. Of 50 patients he observed improvement in 60 per cent and 40 per cent were free of convulsions for twelve years. In a paper published in 1927, Spangler[9] limited the venom treatment to the type of epilepsy in which there is an allergic factor and did not mention his early theory of the effect of venom on the blood coagulation rate. He injected small doses of crotalin solution to

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induce a decreased state of allergy or nonspecific desensitization. He limited the usefulness of venom to those cases in which the patients react to the injections with an increase in eosinophile cells. The eosinophilia following venom injections is said to be an indication of the reactive power of the individual, and in cases in which there is no eosinophilic response improvement is not to be expected. Fitzsimmons[10] in 1929 reported successful results in epileptics treated with a mixture of venoms distributed under the name of "venene."

The favorable results reported from the use of snake venom in epilepsy and the availability of a carefully prepared and standardized snake venom (Moccasin-Mulford) has led me to institute this treatment in a group of epileptics at the Elgin State Hospital. Moccasin snake venom has been used with favorable results in some hemorrhagic conditions.[11] There is no distinguishable difference in the physiologic action of the venom of the water moccasin from that of the rattlesnake.[12]

PROCEDURE

Eight patients with frequent epileptic seizures were selected for this therapeutic investigation. At this point it is appropriate to mention that institutional epileptics differ from extramural epileptics, not only in the fact that the former show mental deterioration but also in that they have organic changes in the brain. Although epileptics in Elgin are not classified with the organic brain disease group, it is a common experience here to find gross brain changes at autopsy, such as marked dilatation of the ventricles.

A study was made of the records of these patients as to their previous frequency of convulsions under luminal therapy and when receiving no treatment. Moreover, they were observed for a week without treatment before instituting venom therapy. Snake venom solution (Moccasin-Mulford) 1:3000 was used. The initial dose was 0.2 c.c. injected intradermally in the upper arm. The succeeding doses were increased by 0.2 c.c. until the maximum dose of 1.0 c.c. was reached. The injections were given twice a week, and after a dose of 1.0 c.c. was reached, the injections were given at weekly intervals. I was guided by the eosinophilic response as to the time of the injection, the venom having been administered when the eosinophilia subsided. The therapy was continued for nine weeks. An attempt was made to discontinue luminal and keep the patients on venom alone, but it was found that the frequency and severity of the convulsions increased to an alarming extent, the patients sustaining numerous injuries during the seizures. It was deemed advisable, therefore, to include 1 ss gr. of luminal, b.i.d. in the treatment. However, the patients were given venom alone, luminal having been omitted for a week at a time in the beginning, middle, and toward the end of the nine-week period of treatment. The definitely unfavorable effects of the venom made it advisable to discontinue this form of treatment after nine weeks. They were again placed on luminal therapy for two weeks and were then observed for a week under no treatment whatever. The results are summarized in Table I.

The seizures were more severe and more frequent during the venom therapy period than on luminal or during the period of no treatment. The

patients were considerably more irritable when under venom treatment than during any other period. The severity and frequency of the seizures after discontinuing the injections of venom, both during luminal treatment and under no treatment, corresponded to the observations made during similar periods before the venom was administered.

The injections of snake venom caused local reactions in the form of erythema and swelling which subsided after cold applications.

TABLE I

PATIENT	AGE AT ONSET (YEARS)	PRESENT AGE	FREQUENCY OF SEIZURES DURING NO TREATMENT PERIOD (PER WEEK)	FREQUENCY OF SEIZURES DURING LUMINAL TREATMENT (PER WEEK, UNLESS OTHERWISE STATED)	FREQUENCY OF SEIZURES DURING TREATMENT WITH SNAKE VENOM (MOCCASIN) (PER WEEK)	HIGHEST EOSINOPHILIC RESPONSE IN PER CENT
H. S.	14	42	4	2 per month	5	8
R. E.	9	30	2	0	5	7
G. H.	9	32	1	2 per month	3	6
J. R.	12	35	4	1	6	8
E. H.	8	21	6	1	8	15
L. K.	16	25	5	1	7	6
M. T.	9	34	3	0	4	3
F. G.	22	28	1	1 per month	8	8

COMMENT

It became obvious after nine weeks of treatment that snake venom therapy is of no value in institutional epilepsy. Instead of inducing a refractory state toward convulsive seizures in patients with epilepsy, it probably renders them more susceptible to fits. This is in agreement with the findings of Thom[6] and contradicts the reports of Spangler and others. Although this experiment was done on institutional epileptics who probably have organic brain changes, nevertheless it may be deduced that the venom does not render a patient refractory to a convulsive seizure viewed as a symptom. The reactivity of the patients to venom injections was well marked as shown by the local reaction as well as the definite response in eosinophilia. There was no correlation between the percentage eosinophilia and the effect of the venom on the frequency of seizures.

SUMMARY

Eight institutional epileptics were treated for nine weeks with snake venom (moccasin). Injections of a 1:3000 venom solution were given, beginning with an initial dose of 0.2 c.c. and increasing by 0.2 c.c. until a dose of 1.0 c.c. was reached. The injections were given twice a week until the maximum dose was reached, when they were administered at weekly intervals. The course of treatment lasted nine weeks. A local reaction in the form of erythema and swelling at the site of injection, and a general reaction evidenced by an eosinophilia were observed. The frequency and severity of the seizures were compared during the periods of no treatment, during luminal therapy and during the administration of venom. During the administration of venom the

frequency and severity of the seizures were greater than during the other periods and the patients were more irritable. There was no correlation between the seizures and the eosinophilic response.

It is concluded that venom therapy not only does not induce a refractory state to convulsive seizures in institutional epileptics but may render them more susceptible to seizures. Although this conclusion is based on experience with institutional epileptics, it probably is also applicable to extramural epilepsy, viewing the convulsive seizures as a symptom.

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DURATION OF IMMUNITY FOLLOWING DIPHTHERIA PROPHYLAXIS*

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IN 1913, Behring[1] demonstrated that the injection of a properly balanced mixture of diphtheria toxin-antitoxin in man would produce no harmful effects and yet produce an antitoxic immunity to diphtheria. Since that time efforts have been made, with more or less success, to prepare products that would engender a better and more lasting immunity.

In 1923, Glenny, Allen and Hopkins[2] proposed that formalinized toxin, or toxoid, be used in human immunization and in 1924 Ramon[3] of the Pasteur Institute perfected such a preparation which, when treated with formalin and heat, completely lost its toxicity but still retained its flocculating and antigenic qualities. It was found to be much more stable than toxin-antitoxin, having greater resistance to heat and demonstrating no apparent change from freezing. One of its chief appeals was that it contained no animal serum, thus avoiding any possibility of serum sensitization following its administration.

From information available two doses of toxoid appear to be from 20 to 30 per cent more effective than three doses of toxin-antitoxin, and the immunity is produced much more rapidly.

In 1926, Glenny, Pope, Waddington and Wallace[4] showed that the antigenic value of diphtheria toxoid was improved by the addition of aluminum potassium sulphate and in 1931, Park and Schroder[5] used alum toxoid for immunization of children with very satisfactory results.

In 1932, Wells, Graham and Havens[6] described the complete precipitation of diphtheria toxoid with alum following the method of Glenny and Barr.[7]

A single injection of the alum precipitate tested in guinea pigs produced such a high degree of immunity that the observations were extended to the effects of a single injection in children.

In later publications, Havens and Gill,[8] Graham, Murphree, and Gill,[9] McGinnes, Stebbins and McCoy,[10] McGinnes, Stebbins, and Hart,[11] Massey,[12] Baker and Gill[13] and others reported on thousands of cases in which a high rapid immunity was produced by one dose of alum precipitated toxoid.

Park[14] introduced it in the New York City Public Schools in 1934, and found that one dose conferred immunity in 95 per cent of susceptible children.

Keller and Leathers[15] state that results following immunization with a single dose of alum precipitated toxoid compare favorably with those obtained after two doses of toxoid and are much better than those after three doses of toxin-antitoxin mixture.

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DURATION OF IMMUNITY

On the basis of guinea pig experiments, carried on for a long period of time, it was presumed that in human subjects an immunity equal to or superior to that obtained with regular toxoid could be obtained with alum precipitated toxoid.

Gill, quoted by Walker,[16] reported that in a retest of forty of his original group of children made two years later, there was one that gave a slightly positive reaction; all the rest were negative. All of these children were strongly Schick-positive before receiving the alum precipitated toxoid, and all had reacted negatively after injection.

McGinnes, Stebbins, and Hart[11] reported on the durability of immunity produced by one dose of alum precipitated toxoid at the end of one year (Table I).

TABLE I

TABLE FROM MCGINNES, STEBBINS, AND HART
Am. J. Pub. Health 24: 1141, 1934

REVERSION OF SCHICK NEGATIVES IN ONE YEAR			
	POSITIVE	TOTAL	PER CENT SHOWING REVERSION
Originally Schick-negative With no history of previous immunization	36	550	6.5
Originally Schick-negative With history of previous immunization	24	519	4.6
Originally Schick-positive Rendered Schick-negative by Park's toxoid	10	225	4.4
Originally Schick-positive Rendered Schick-negative by alum ppt. toxoid	19	342	5.6
	89	1,636	5.4

Their conclusion is that there is not a significantly greater reversion to the Schick-positive state in individuals rendered Schick-negative by alum precipitated toxoid than in (Schick-negative) individuals rendered Schick-negative by natural causes or following other diphtheria immunizing agents.

Farago[17] conducted some experiments in Hungary with a single dose of alum precipitated toxoid. Of a group of 2,652, 93.3 per cent were Schick-negative after two months. One year later 2,379 of this same group were again Schick-tested and 93.7 per cent were negative.

TABLE II

SUMMARIZED TABLE OF FARAGÓ'S PAPER
F. Faragó: Am. J. Hyg. 22: 495, 1935

IMMUNIZED SCHICK CHILDREN		IMMUNIZING DOSE LF UNITS	RESULTS OF SECOND AND THIRD SCHICK TESTS			
			AFTER TWO MONTHS		AFTER ONE YEAR	
AGE YEARS	NUMBER		NUMBER PRESENT	SCHICK NEGATIVE	NUMBER PRESENT	SCHICK NEGATIVE
2-12	4,297	14.4 to 29.4	2,652	93.3%	2,379	93.7%

Faragó states that the total number of children in Hungary immunized with toxoid precipitate, up to the end of 1934, was about 60,000, that it is

now possible to assert that immunization with toxoid precipitate is a simplified means of fighting diphtheria, and that in his experience it confers as great a protection as that produced by the Ramon toxoid.

Recently a pamphlet was distributed on the *Detailed Study on Diphtheria Immunization With Alum Precipitated Toxoid* by H. H. Pansing, Health Commissioner of Montgomery County, Dayton, Ohio, and E. R. Shaffer.[18] One group of 462 children, given one dose of 1 c.c. of alum precipitated toxoid, and re-Schicked twenty-eight days later, showed 388 or 84 per cent negative. The 74 Schick-positive children were retested after sixty days and seven were negative, giving a total of 85.5 per cent negative.

A second group of 495 Schick-positive children were given a single dose of alum precipitated toxoid. Approximately sixty days later 445 of these were re-Schicked and 363 or 86 per cent were found negative.

A third group consisted of children from the two groups, all of whom were Schick-negative twenty-eight or sixty days following administration of one dose of alum precipitated toxoid.

Of this group 549 were again Schick-tested two years after their toxoid vaccination and of these 317 or 57.8 per cent had reverted to Schick-positive.

The children who were Schick-positive sixty days after their original dose of toxoid were given a second injection. Of this group 105 were Schick-tested two years later and 43 or 40 per cent had reverted to Schick-positive.

TABLE III

SUMMARY FROM PANSING AND SHAFFER'S PAMPHLET

GROUP	ALUM TOXOID	NUMBER CHILDREN	SCHICK TEST AFTER SIXTY DAYS	
			NUMBER	SCHICK NEGATIVE
A	1 c.c.	468	462	85.5%
B	1 c.c.	495	445	86.0%
				SCHICK POSITIVE
C	After two years		549	57.8%
	(Two doses toxoid)		105	40.0%

Pansing and Shaffer conclude that the evidence obtained indicates that at least three doses of any diphtheria antigen of choice are required to establish a reasonable degree of permanency.

The object of this paper is to report on the results offered in a small series of our own. A large institution in the state of Indiana with approximately 500 children between the ages of seven and twenty, and 100 adults (teachers, supervisors, kitchen help, laundry, etc.) had been having numerous cases of diphtheria each winter. In 1928 they had a particularly severe epidemic and our aid was requested.

Dr. E. G. Stewart and Jones Schick-tested and made throat cultures of every one in the institution. All the Schick-positives were made Schick-negatives by administration of toxin-antitoxin mixture.

Each year since that time (with the exception of 1933) all of the new inmates were Schick-tested and Schick-positives, treated with some form of diphtheria immunizing agent, and re-Schicked to prove establishment of immunity. Since none of them had been re-Schicked for a considerable period

of time the opinion was held, after reading Pansing and Shaffer's paper, that this would be desirable to see what information on the development of immunity they would yield.

Of the group of children who received toxin-antitoxin in 1928, 121 were still in the institution (1936). These were re-Schicked and 6 individuals or 5 per cent were again positive.

Of 13 children who were Schick-negative in 1928 and who were not given any toxin-antitoxin, 3 individuals or 23 per cent were positive.

In 1929, several reports were published on the production of immunity to diphtheria by inunction of the toxin-antitoxin in a lanolin or jelly base.

A number of Schick-positive children were given five inunctions in a lanolin base. The response was weak. Three months later five more inunctions in a jelly base were applied. Through an oversight a re-Schick was not made. Thirty-five of these children are still in the institution and were Schick-tested. Fifteen or 43 per cent were still positive.

During 1930, 1931, and 1932, all Schick-positive children were made Schick-negative with diphtheria toxoid. Of those remaining:

21 from 1930, 3 or 14.3 per cent were again positive.
28 from 1931, 2 or 7.1 per cent were again positive.
30 from 1932, 2 or 6.7 per cent were again positive.

Ten children from 1931 and eleven from 1932 who were negative at that time and received no toxoid, are still negative.

In 1934 we started giving alum precipitated toxoid; 18 were still in the institution and after two years all are Schick-negative.

Thirteen who were negative in this year and received no treatment are still negative.

In October, 1934, we again used alum precipitated toxoid on the new pupils who were Schick-positive. Forty-four children were tested and 31 were found to be Schick-positive. These were divided into two groups: 16 were given one dose of 0.5 c.c. containing 25 Lf units. The remaining 15 were given one dose of 0.5 c.c. containing 15 Lf units.

These children were again tested four weeks after the alum toxoid. Of the children receiving 25 Lf units, two or 12.5 per cent were still Schick-positive; of those receiving 15 Lf units, 3 or 20 per cent were still Schick-positive.

All of these children were again tested this year (sixteen months after one injection of alum precipitated toxoid). Two were found to be Schick-positive. One of these was a child who was Schick-positive last year on retest after receiving 15 Lf units and therefore is not a reversion. The other four children who were still Schick-positive four weeks after the injection of the alum toxoid are now Schick-negative.

The other positive reaction was a slight reaction, 13 mm. in diameter, in a child who received 25 Lf units and was Schick-negative four weeks after inoculation.

Thirteen untreated children who were Schick-negative during October, 1934, are still negative sixteen months later. At this time (February, 1936) 70 new students have been Schick-tested. Twenty are positive and will be inoculated with one injection of alum precipitated toxoid.

TABLE IV

REVERSION OF SCHICK NEGATIVES OVER A PERIOD OF EIGHT YEARS

	POSITIVE	TOTAL	PER CENT REVERSION
8 years previously—			
Immunized with toxin-antitoxin mixture	6	121	5.0
No history of previous immunization	3	13	23.0
6 years previously—			
Immunized with toxoid	3	21	14.3
4 and 5 years previously—			
Immunized with toxoid	4	58	6.8
No history of previous immunization	0	21	0.0
16 to 24 months previously—			
Immunized with alum toxoid	1	49	2.0
No history of previous immunization	0	26	0.0

In this series of approximately 428 children a Schick control test was made along with the Schick test.

It is interesting to note that 47 or 11 per cent gave positive pseudoreactions. Of these, 10 were positive-combined and 37 were negative Schick.

In subgrouping these 47 we find:

18 had no record of previous injections of toxoid or toxin-antitoxin.
22 had been inoculated with toxin-antitoxin mixture.
2 had been inoculated with toxoid.
5 had been treated with ointment.

While 11 per cent of the whole group gave pseudoreactions, all of those who reacted were over thirteen years of age. This would indicate the importance of a Schick control test in all individuals over twelve or thirteen years of age.

CONCLUSIONS

While the number of retests are small, the result would agree with those of Gill, and McGinnes, Stebbins and Hart, and Faragó, indicating that alum precipitated toxoid is a better immunizing agent than toxoid; and toxoid, in turn, is better than toxin-antitoxin mixture and that this immunity is just as lasting after alum precipitated toxoid.

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ROULEAU FORMATION*

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IT SEEMS that rouleau formation was first noted by "Dr. Thomas Hodgkin and Joseph Jackson Lister in 1827." Both of these names are known in medicine. J. J. Lister was the father of the late Lord Lister, who introduced aseptic surgery, and Thomas Hodgkin first described the disease which bears his name. "To those observers the microscope revealed the fact that if a minute drop of human blood is placed between two plates of glass, the red corpuscles apply themselves to each other by their concave surfaces in such a manner as to form long cylindrical masses which resemble piles of coins and that very frequently those piles are so arranged as to form with each other a complete network of rouleaux with clear intervening spaces occupied by liquor sanguinales."[1]

It is evident that some force must operate to bring the corpuscles together and that the same force or an entirely different set of forces must complete and maintain the rouleau formation after contact between corpuscles has once been made. The last part of the suggested analysis has received considerable attention but no explanation or suggestion has been found in the literature as to the causation of the bringing-together force, except for vague references to the existence of a force of attraction and the analysis of chance encounters.

Surface tension, adhesion, cohesion, and similar physical agencies operate only as contact is made and exert no appreciable force between discrete particles suspended in a liquid. The outer surface of an erythrocyte appears to

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be lipoidal in nature and probably is not thoroughly wet by the blood plasma. The fact that erythrocytes do not adhere in the normal blood stream indicates that the surface tension effect is not very pronounced, since corpuscles frequently come in contact with others.

The forces due to surface tension, when they operate on two erythrocytes already in contact, undoubtedly do have a tendency to place them face to face; this is the position of least overall surface.[2] But if a pile of corpuscles has already formed in rouleau, it would be anticipated that a considerable proportion of future additions to the pile would become attached to the sides if the contacts were entirely adventitious. Observation shows that this rarely happens. It is accordingly apparent that a force operates within the liquid to place the free erythrocytes on the ends of the rouleau piles which are already formed.

Erythrocytes are only about 5 per cent heavier than the blood plasma which they displace. Their movements accordingly respond to very delicate forces; they are like feathers floating in air. Also, the erythrocytes compose about one-fourth of the volume of the blood, and they are accordingly relatively numerous and only slightly separated from one another.

The formation of the rouleaux appears ordinarily to take place just as the cover glass settles into position before the blood coagulates. The placing of the cover glass on the drop of blood causes a relatively strong current of blood to flow. The piling of red blood corpuscles into rouleau formation may be greatly increased by pumping the blood back and forth under the cover glass by means of intermittent pressure, such as could be exerted with a pencil point. Streams of the viscous plasma pass about the corpuscles whenever the conditions of movement are changed or reversed, because of the greater inertia of the corpuscles which are slightly heavier than is the plasma. Hydrodynamic reasoning indicates that the distribution of the velocity of the moving plasma is a maximum halfway between the plates. Further, and particularly important, the corpuscles which are immersed in the moving liquid tend to occupy the center of the stream and assume a position of maximum stability, which is with their plane surfaces at right angles to the stream flow, i.e., with their axes of symmetry parallel to the stream flow. This is the condition of maximum stability as was shown by the physicist Lord Rayleigh who mathematically analyzed the conditions of flow about a disk in a stream of viscous fluid. He showed that the internal friction of the fluid as it flows around the edges of the disk causes a couple to be set up. The torque exerted by the couple places the plane of the disk at right angles to the stream flow. All the discoid corpuscles tend to do this and accordingly there is an orderly procession of them with their broad sides parallel; and if they come together at all, it is always in this broadside-parallel fashion. The relative motion between the corpuscles and the plasma is such that the resistance of the flow of the liquid about the erythrocytes is decreased after the rouleaux have formed. These are then the causes which set up the formation of rouleaux. Hydrodynamic forces operate to bring the corpuscles together with their faces parallel and the forces of surface tension complete the positioning of the corpuscles and hold them together.

Richard Norris from whom a previous paragraph was quoted produced the phenomenon of rouleau formation in 1868 with water and weighted cork disks, which had been paraffined. The author has used disks 8 mm. in diameter and about 1 mm. thick made of carrots to study the phenomenon. Rouleaux were produced by placing a goodly number of these disks in about a centimeter of kerosene in a flat-bottomed dish and gently shaking them. In such experimentation, it is readily apparent that the relative motion of the liquid and the disks exerts a great influence in bringing the corpuscles together.

It has been shown also that small light particles in a medium which moves at a different velocity than they do sometimes exhibit a quite striking and even similar phenomenon to rouleau formation. See, in almost any textbook of physics, descriptions of the Kundt tube method of determining the velocity of sound. Very light particles of cork dust are made to pile up in discrete masses in a tube by the induced air currents in such a way that the wave lengths of sound are readily apparent. Also, Bergen Davis has noted that light cylindrical particles, like the capsules which are used in powder medication, arrange themselves end-on in transverse rows across a horizontal organ pipe when it is sounding.[3]

SUMMARY

Rouleau formation of erythrocytes between a cover glass and a glass slide has been explained on the basis of hydrodynamic principles. A means is pointed out by which enhancement of the phenomenon is possible. A method for artificially producing rouleau formation with large plainly visible discoid particles is described.

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HUMAN INFECTION WITH MONILIA*

Report of a Case With Cultural Data

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PATHOLOGIC processes in the human body associated with monilia are not rare, but the unusual lesions produced in this case are of interest because of the confusing similarity which caused diagnoses of syphilis, tuberculosis, and leprosy to be made before the true causative organism was found. The closed subcutaneous and the open pulmonary lesions in this case afforded an unusual opportunity for cultural studies.

E. R., a colored male, aged forty-one years, was admitted to the National Leprosarium, July 12, 1934, with a diagnosis of leprosy which could not be confirmed; therefore a study of his case was begun.

The patient was born in Virginia but had spent most of his life in Ohio. His occupations had been chiefly manual labor. The previous and family histories were not remarkable.

Previous Illnesses: Chickenpox at the age of four years with good recovery. Smallpox at the age of sixteen with good recovery.

About 1920 a tumor appeared on the upper lid of the left eye about the size of the end of the small finger. About two weeks after this was aspirated with a syringe, a burning of the eye was noticed, and at this time the patient noticed his vision was beginning to be blurred. A discharge continued from the eye and in spite of medical treatment the vision continued to be blurred and the eye went totally blind about 1930. At the present time the vision of the right eye is periodically blurred. The patient gave a history of having had double pneumonia in 1924 with poor recovery. The patient said he had never felt well after the attack and had retained a residual cough. Slight improvement was noted following a period of rest. During this attack of pneumonia the patient lost 23 pounds of weight, some of which was regained but he was unable to hold it. He has not gained enough strength since to do manual labor. Several years ago the patient said he had night sweats for about one week but had not been troubled with them since.

In 1924, the patient stated that soft subcutaneous tumors appeared on the anterior surfaces of both wrists, which had gradually enlarged. In 1930 small raised nodules appeared in the skin of the face, one at a time. This same year one of these nodules was removed surgically and shortly afterward several appeared within the same area. These lesions have remained practically the same until the present time.

In 1927, the patient gives a history of mashing his right index finger with a wrench and crushing the bone. Recovery was fairly good. Three years later the finger became swollen and reddened at this place. No pain was associated with the swelling at any time. It has persisted and gradually become shortened.

The patient gives a history of having had gonorrhea at the age of eighteen years and a "haircut" on the penis in 1918 but denied secondary manifestations of syphilis.

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The present illness dated back ten years by the patient to the attack of pneumonia. The chief complaints at the time of admission were pain in the chest, hoarseness, and cough.

Physical Examination: The patient was emaciated, weighed 130 pounds (peak weight had been 165 pounds), was extremely hoarse and showed considerable dyspnea. The temperature range was 37° to 38° C. and the pulse from 100 to 120.

The face showed small papules and nodules on the cheeks, nose, lips, forehead, and eyelids. These ranged in size from 0.2 to 1 cm. in diameter and showed a tendency to mass together. The tops were excoriated and many showed umbilication with depressed centers. Some of these lesions showed redness while others showed practically no color change except the excoriated tops. The lesions were fairly firm and showed no tendency to discharge pus, although in some of the excoriated tops there was evidence of superficial secondary infection,

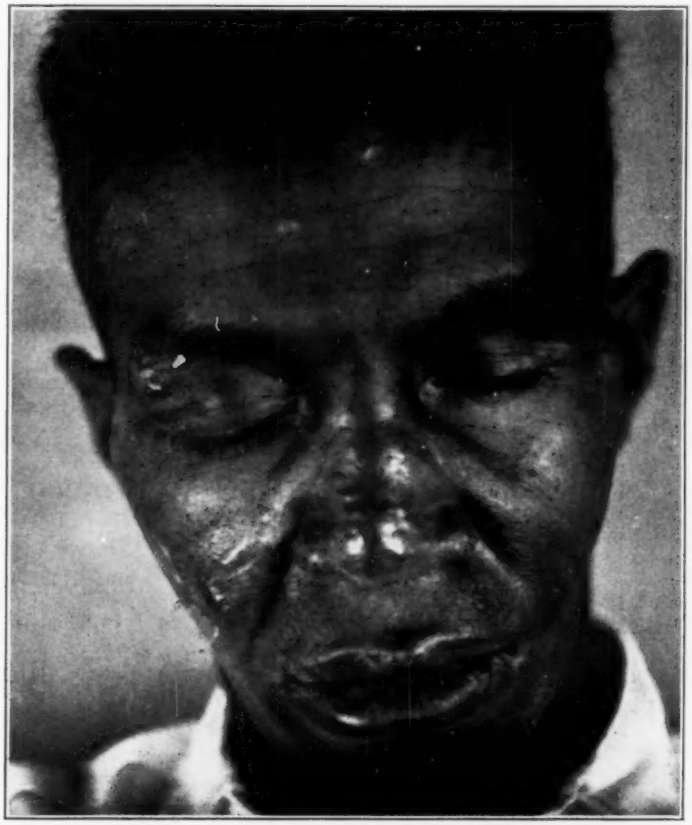

Fig. 1.—Photograph of Patient E. R., showing nodular and papular lesions of the face.

but in these instances they were dry and crusty. The left cheek was free from these lesions but showed several small lesions of dermatosis papularis nigra. The skin of the rest of the body was free from lesions, except for a few leucodermic areas on the anterior chest.

A generalized lymphadenopathy was present. Both wrists showed soft subcutaneous tumors 2 to 4 cm. in diameter on the anterior surfaces which appeared to be connected with the tendon sheaths.

The right index finger was swollen and red in color. The two terminal phalanges were shortened. The nail was attached but deformed. The skin was excoriated at the juncture of the proximal and middle phalanges, and the surface was moist, although no frank pus could be expressed. The terminal phalanges of the right middle and left index fingers were "crooked."

A few firm subcutaneous nodules not over 1 cm. in diameter could be felt on the anterior surface of the left forearm. There was a small scar on the glans penis. A right inguinal hernia was present. The left eye was blind and the conjunctiva of the right eye was reddened. The heart was very rapid on slight exertion. Both lungs showed bronchovesicular breathing throughout. No râles could be heard. The physical examination was otherwise negative.

X-ray Examination: Lungs: Both showed dense shadows at the roots and shadows characteristic of fibrosis extending to the periphery involving all lobes. The picture was not unlike that of chronic tuberculosis. Hands: The distal and middle phalanges of the index finger of the right hand showed advanced absorption of the bone with only a slight amount remaining. The end of the proximal phalanx of this same finger showed osteoporosis and erosion. There was a slight amount of erosion of the middle phalanx of the middle finger. Similar changes were present in the middle and ring fingers of the left hand.

Laboratory Examinations: Urinalyses were negative except for a few granular casts. The blood counts were not remarkable. Kolmer-Wassermann tests were 4-plus and the Kahn test was 4-plus.

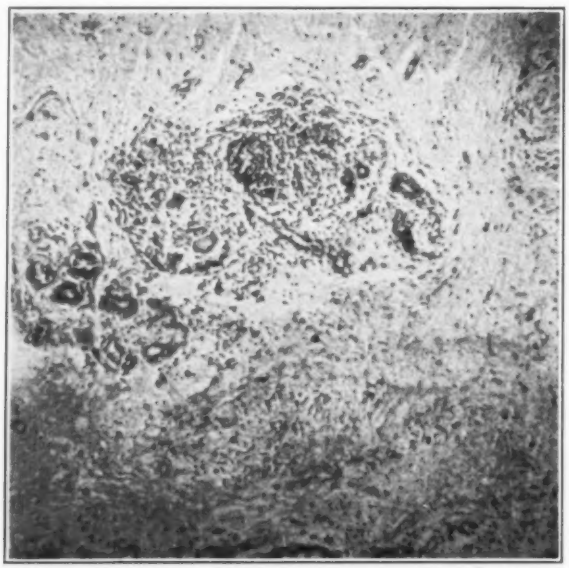

Fig. 2.—Photomicrograph of a nodule on the face, showing an area of round cell infiltration with a foreign body giant cell around a sweat gland (low power).

The sputum and nodules on the face were searched numerous times for acid-fast bacilli but none were found. Concentrated specimens of sputum were negative for acid-fast bacilli as were guinea pig inoculations and cultures on Petroff's medium. The sputum was purulent in character and blood streaked.

While searching the sputum for acid-fast bacilli frequent yeastlike organisms were seen in the smears. These cells were found to be a constant finding. A similar organism was then found in smears prepared from the papules of the face. One of the soft subcutaneous tumors of the wrist was removed and a similar organism was found to be present. This tumor consisted of a rather soft lobulated mass 2 cm. in greatest diameter, grayish yellow in color, very moist but containing no pus. Cultures of the yeastlike organism were obtained on Sabouraud's medium from the sputum and the tumor from the wrist and will be described in detail. Dark-field examinations of the sputum for spirochetes were negative in each instance.

Histologic sections prepared from the tumor removed from the wrist showed myxomatous type tissue composed of spindle cells and very loose fibrils with spaces between them. Interspersed with these fibrils were areas of scanty infiltration by lymphocytes and a few monocytes.

Sections prepared from a nodule removed from the face showed slight pressure atrophy of the epidermis. The corium showed numerous circumscribed areas of infiltration of lymphocytes and monocytes with an occasional foreign body giant cell. There was slight fibroblastic reaction. There was no necrosis.

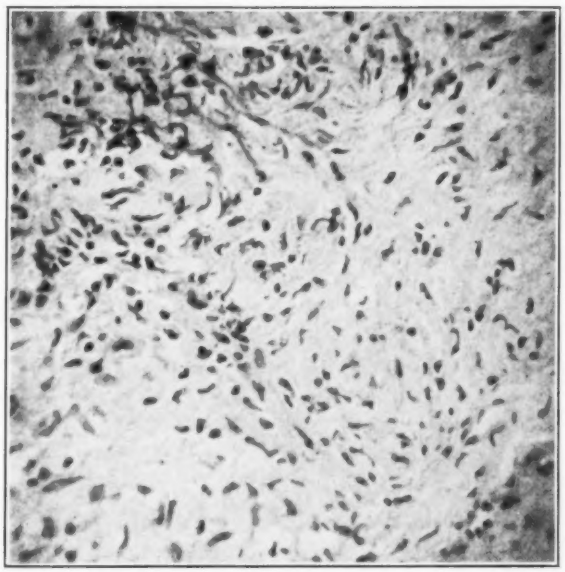

Fig. 3.—Photomicrograph of a subcutaneous tumor near the wrist, showing its myxomatous nature (high power).

Fig. 4.—Photograph of a single colony of monilia isolated from the patient and growing on starch agar. Submerged mycelium growing out from smooth, moist surface colony.

Morphologic and Cultural Characteristics: In smears made from fresh lesions from inoculated animals and stained with methylene blue or preferably with Wright's stain, the organisms appeared often in large numbers as rounded or slightly oval cells of varying sizes, but averaging about 8 microns in diameter. There was a heavy refractile, usually unstained

capsule around the parent cell and a thinner one around the budding cells. The cells stained very irregularly, some showing many dark staining granules or masses and some almost unstained.

In cultures on Sabouraud's or dextrose tartaric acid agar, the growth was luxuriant, pearly white, moist, smooth, and rounded in outline. As the culture became older, there was a tendency for it to become dry, wrinkled, and slightly cream colored. After the organism had been isolated for several months, filaments were seen projecting from the undersurface of the colony into the media in cultures a week or more old. No aerial mycelium was produced. The original cultures made from the patient required about three days at 37° C. to develop. Later the organism grew luxuriantly in twenty-four hours at 37° C.

Microscopically, the cells in young cultures looked much as they did in tissue; the majority were oval or rounded, about 8 microns in diameter, giving off smaller budding forms. They varied in size depending to some extent on the moisture of the culture. Usually there was only one bud to a cell but as many as three or four were sometimes seen.

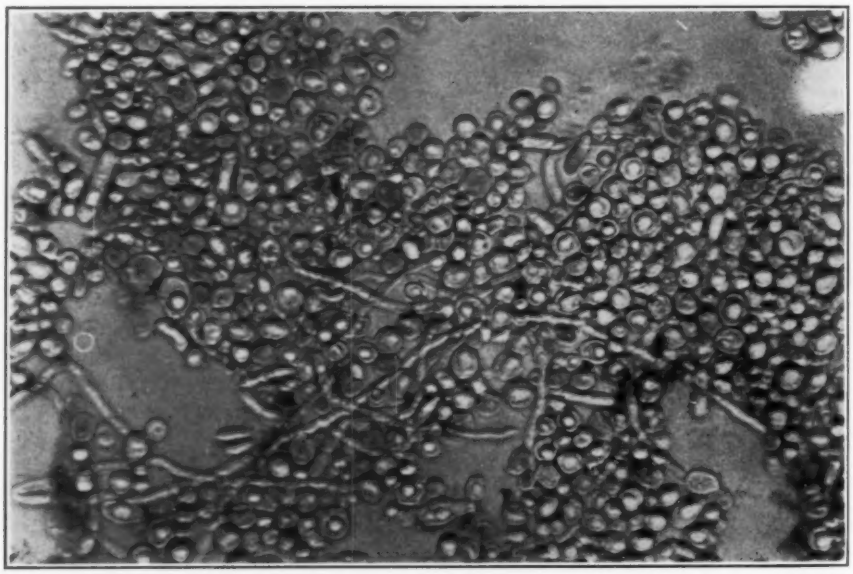

Fig. 5.—Photomicrograph of a smear from the culture of monilia showing budding and mycelium formation (×600).

In older cultures, on some media more than others, septate mycelia were seen giving off lateral branches and buds and terminal buds. Very occasionally terminal chlamydospores were seen. Budding cells predominated even in smears from old cultures.

The structure of the cells could be best seen in preparations mounted in 3 per cent sodium hydroxide solution. The rounded or oval cells appeared as typical yeast cells. The cells were surrounded by a thick capsule and the budding cell by a thinner one. Vacuoles were numerous in most of the cells.

Preparations stained with 1 per cent neutral red showed the red dancing body and pink granules of typical yeast cells. Fat in the vacuoles was demonstrated by staining with Sudan III. The organism was gram-positive when stained by Gram's method, and some cells contained granules more intensely stained than the rest of the cell. Some cells were stained reddish brown with Gram's iodine, others were almost unstained.

A small irregular nucleus usually at one side of the cell could be seen when the organism was stained by Mallory's chloride of iron hematoxylin method.

An acid reaction of the medium was most favorable. Growth on nutrient agar of pH 7.0 was less luxuriant than on an acid medium of pH 5.0 to 3.8. When the sputum and tumor were cultured on Petroff's medium, no growth occurred although future transplants showed growth on this medium.

In bouillon the organism grew in the bottom of the tube as a scant granular sediment when first isolated. Later, it grew more abundantly as a coarsely flocculent mass near the surface, which soon sank to the bottom and became granular.

In 1 per cent peptone in half molecular soluble phosphate solutions, Draper[1] found that *Oidium albicans* produced abundant mycelium. This organism, however, in 1 per cent peptone in one-half molecular sodium acid phosphate solution grew only as a granular sediment on the bottom and sides of the tube. Microscopically, the round or budding cells predominated, but there were a few mycelial forms giving off budding cells on the ends. Growth in milk was poor. Lead acetate agar was blackened. Litmus was not reduced. Indol was not produced.

Gelatin was not liquefied and growth in this medium was scanty. No filaments radiated out from the stab line and no arthrospores were seen when the organisms were examined microscopically.

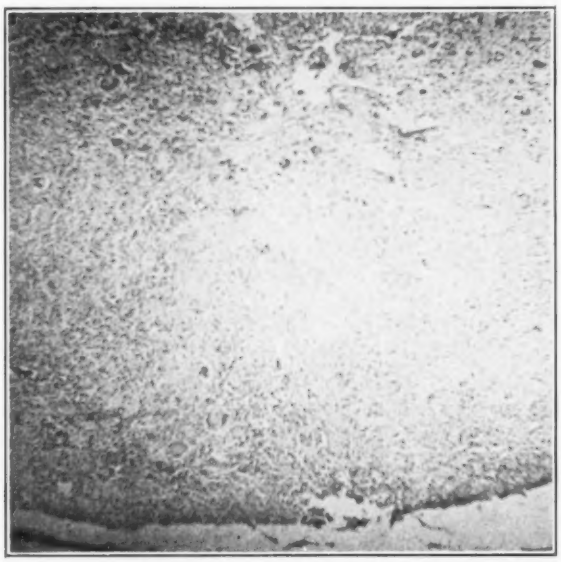

Fig. 6.—Photomicrograph of an inflammatory tumor mass of the omentum of a rat produced by an intraperitoneal injection of the monilia. Note foreign body giant cells (low power).

Fermentation reactions were tested by growing the organism in 1 per cent peptone and 0.8 per cent sodium chloride solution, containing 2.5 per cent of the different carbohydrates, glucosides, or alcohols with brom thymol blue as an indicator. The results are given in Table I. The acid was later utilized, for after a time the reaction became alkaline. Gas was produced in abundance, usually 60 to 75 per cent, in d-glucose, d-fructose, d-mannose, maltose, and trehalose and was mostly carbon dioxide. On some of the sugars a pellicle occurred on the surface of the media; in others, most of the growth took place at the bottom of the tube. The latter was true particularly of the sugars which were fermented with the production of acid and gas.

Cellulose and starch were not utilized. Filter paper moistened with dextrose peptone solution and inoculated with the organism was not softened. Starch was tested by growing the organism on starch agar and flooding with Gram's iodine. Submerged mycelia were produced abundantly from colonies on starch agar. In a few days the feathery mycelia could be seen projecting into the agar from the surface of the round, smooth, moist surface colony.

No ascospores were seen in preparations made from twenty-four-hour cultures on Gorodkowas medium in either glass or clay petri dishes at 20° C., 31° C., or 37° C., or in preparations made from cultures on any other media.

Scant growth was obtained on Sabouraud's medium, and dextrose tartaric acid medium made anerobic either with or without the presence of carbon dioxide. More mycelial forms were seen under the microscope than in similar aerobic cultures, but the rounded or oval budding forms were also seen.

Growth was more luxuriant at 37° C. than at room temperature. Microscopically there was no difference in the morphology of the organism, whether it was incubated at room temperature or at 37° C.

Pathogenicity: The organism was pathogenic for rabbits, rats, guinea pigs, and mice. A suspension from a twenty-four-hour-old culture on dextrose tartaric acid medium was made in 0.85 per cent sodium chloride solution and 1 c.c. was injected intravenously into a rabbit, 1 c.c. intraperitoneally into a white rat, 1 c.c. subcutaneously into a guinea pig, and 0.5 c.c. subcutaneously into a mouse.

TABLE I

FERMENTATION REACTIONS OF MONILIA ISOLATED FROM PATIENT E. R.

CARBOHYDRATES, GLUCOSIDES OR ALCOHOLS	ACID	GAS
d-Glucose	+	+
d-Fructose	+	+
d-Mannose	+	+
d-Galactose	+	?
l-Xylose	+	–
l-Arabinose	+	–
Rhamnose	+	–
Maltose	+	+
Trehalose	+	–
Saccharose	–	–
Lactose	+	–
Aesculin	+	?
Dextrin	+	?
Salicin	+	–
Melezitose	–	–
Melibiose	–	–
Raffinose	–	–
Glycerol	–	–
Adonitol	–	–
Inositol	–	–
Dulcitol	+	–
d-Mannitol	+	–
d-Sorbitol	+	–

NOTE: + indicates positive, – indicates negative, and ? indicates a trace.

The rabbit died in two days. The liver presented a few well-defined abscesses filled with yellow pus and the kidneys were slightly mottled in appearance. The other organs showed no definite lesions. Smears made directly from the heart, blood, lungs, spleen, liver, and kidney showed the yeastlike organism, while the liver and kidney also showed the presence of *Eimeria stiedae* which no doubt accounted for the lesions. Smears from the meninges were negative.

The organisms were obtained on cultures made on Sabouraud's and dextrose tartaric acid media from the liver, spleen, heart blood, and kidney. A suspension made from the culture of the organism from this rabbit was injected into a second rabbit. The second rabbit died in three days and the findings were the same. Lesions in the kidney and liver were accounted for by finding *Eimeria stiedae*. Direct smears showed also the presence of the yeastlike organism in budding form in the heart blood, peritoneal fluid, lungs, kidney, and liver. Cultures from several organs were obtained on Sabouraud's and dextrose tartaric acid media. The meninges appeared normal and no yeastlike organisms were seen on direct smear.

The rat died nineteen days after intraperitoneal inoculation. A yellowish mass in the omentum about 1.5 by 1 by 1 cm. adhering to the pyloric end of the stomach was found.

Oozing from it was a thick pus. The lungs were hemorrhagic and there was a small yellowish abscess in one testicle. Direct smears made from the liver, omentum, spleen, kidney, adrenal, lung, and testicle showed the organism and cultures were obtained on Sabouraud's medium and dextrose tartaric medium from the liver, spleen, testicle, omentum, lung, and kidney.

A second rat injected intraperitoneally with a culture of the organism from this rat died in six days, much sooner than the first, but otherwise presented much the same lesions. The mass found in the omentum was the same. There was no abscess in the testicle but there were a few in the liver. The organism was seen in direct smear from several of the organs and was also obtained in cultures.

The guinea pig died eleven days after subcutaneous inoculation. Subcutaneously at the site of injection a soft yellowish mass about 1.3 by 1 by 1 cm. was found and a smaller mass in the omentum near the stomach. The lungs were edematous and the adrenals were enlarged. No yeastlike organisms were seen in direct smears made from these organs and stained with methylene blue but cultures of them were obtained from the lung and subcutaneous mass on Sabouraud's medium. This culture was injected subcutaneously into a second guinea pig, which died in nineteen days and presented almost the same findings. The anterior abdominal wall showed a crust-covered hypertrophied lesion 2 cm. in diameter from which a thick, creamy pus could be squeezed. This extended through the abdominal wall causing a small lesion in the peritoneum. Two slightly enlarged lymph glands were found near this lesion. Near the pylorus of the stomach a mass of omentum about 1 cm. in diameter was found. In the center of this mass was a small grayish lesion about 1 mm. in diameter. Smears made from the subcutaneous lesion showed the presence of the yeastlike organism, but none were found in the smear from the lesion in the omentum. No cultures were made.

The mouse did not die until thirty-seven days after injection. A small subcutaneous abscess was found at the site of the injection. The inguinal glands were enlarged and the kidneys were edematous. A smear from the pus in the abscess revealed diplococci and the yeastlike organisms and smears from the gland, heart blood and kidney showed the yeastlike organism. Cultures made on Sabouraud's and dextrose tartaric acid media were negative for the yeastlike organism.

A suspension of the original culture from the patient was injected subcutaneously into a second mouse. This mouse died in twenty-nine days. A thickened and reddened mass of the omentum was found near the stomach. No other evidence of infection was found. Smears made from the omentum showed the presence of the yeastlike organism, and cultures were secured on Sabouraud's and dextrose tartaric acid media.

Histology of Lesions in Guinea Pig and White Rat: Sections made from the lesion at the site of injection in one guinea pig showed large abscess formation, consisting of necrosis and diffuse infiltration of neutrophiles, lymphocytes, and monocytes adjoined by a rather loose proliferation of connective tissue, consisting of rather faintly stained elongated nuclei and loosely woven fibrils. The scanty leucocytic reaction in this portion of the section consisted chiefly of lymphocytes and monocytes.

Sections made from the mass in the omentum of one rat showed a large area of necrosis accompanied by a diffuse leucocytic infiltration of neutrophiles, lymphocytes and monocytes, almost completely surrounded by a loose connective tissue proliferation similar to that seen in the guinea pig. This connective tissue was studded with numerous foreign body giant cells and showed a scanty infiltration of leucocytes, chiefly lymphocytes and monocytes.

Classification: Henrici[2] says: "The classification of the fungi imperfecti is very unsatisfactory. Undoubtedly in a large proportion of them the imperfection lies in our knowledge of their life cycles." The clinical features of the case, the occurrence of subcutaneous tumor masses, the cutaneous and lung lesions are similar to the infection described by Gilchrist[2] as caused by *Blastomyces dermatitidis* or *Oidium dermatitidis.*

Culturally the organism did not resemble it. It did not produce a tough growth on solid media characteristic of *Oidium dermatitidis,* and it did not produce aerial mycelia. *Oidium dermatitidis* does not ferment any carbohydrates; this organism fermented several carbohydrates with the production of acid and gas.

Michelson,[3] however, reports two cases of systemic blastomycosis in which only yeast forms were obtained on culture.

The pathogenicity of the strain isolated was not of much value in classifying it. Spring[4] reports in a study of seven strains that blastomyces is more pathogenic for mice than for rabbits, guinea pigs, and rats; this organism was less pathogenic for mice. Smith[5] states that *Monilia psilosis* produces a fatal septicemia in rabbits in twenty-four to seventy-two hours. This organism also did but the fermentation reactions were not in accord with any of the strains as given by Smith.

The organism appeared to attack the omentum most frequently but apparently a predilection for the omentum of an animal is not a characteristic of any particular fungus. Spring found involvement of the peritoneal surface in many of the animals injected with *Blastomyces dermatitidis*. Nodules were found by Rewbridge, Dodge, and Ayers[6] in the omentum and the peritoneal surface of mice injected with a strain of *Endomyces capsulatus* isolated from a meningitis case. Nodules were produced almost invariably in the omentum of white rats injected with *Phialophora verrucosa* from a case of chromoblastomycosis by Wilson, Hulsey and Weidman.[7] Connor[8] reports small tubercular lesions in the omentum of mice by a monilia from a case of osteomyelitis. Benham[9] produced gelatinous nodules in the omentum, spleen, and lungs of white rats by injecting *Cryptococcus hominis*.

Although the names Oidium and Monilia are used interchangeably in the literature the differentiation is usually made on the basis of reproduction. The oidia give rise to new cells by a breaking up of the mycelia, giving the appearance of chains; the monilia give rise to new cells by means of budding from the mycelium. By such a basis of differentiation the present organism is a Monilia. This corresponds to the Blastosporinae in Vuillamin's classification.

It produced mycelia scantily as distinguished from fungi which produce them abundantly. No aerial mycelia were observed. This characteristic since it did not produce a heavy dry pellicle on liquid media would place it as a Parasaccharomyces according to Anderson.[8]

It belongs to the fermenting group as distinguished from the nonfermenting.[10] It might be classed as *Monilia pinoyi* according to Castellani's[11] classification of Monilia based on the production of gas from carbohydrates, although it sometimes produced a trace of gas from galactose and dextrin. Amygdalin and erythrite were not tested. As far as production of gas from carbohydrates was concerned it was also similar to a monilia isolated by Smith and Sano[12] from a case of meningitis. In addition to some of the carbohydrates used by Castellani, they employed mannose which their strain of Monilia fermented with the production of gas. It also agreed in the production of gas with the fermentation reactions of a monilia isolated from a case of osteomyelitis by Connor.[8]

The present organism could be called a Monilia, Blastosporina or Parasaccharomyces. The name Monilia appears to be most widely used.

COMMENT

That this case was proved to be an infection with monilia by cultural and animal experimentation there seems to be no doubt. Such conditions are probably not as rare as the number of diagnoses would lead one to believe.

In this case the signs and symptoms, loss of weight, cough, blood-streaked sputum, characteristic x-ray findings, and permanent hoarseness pointed to pulmonary tuberculosis. The absence of acid-fast bacilli in the sputum by smear, culture, and animal inoculation, and the presence of monilia in smears and culture which were pathogenic for ordinary laboratory animals seem to be conclusive proof that an infection of the lungs with monilia was present.

Serologically, the patient was syphilitic as evidenced by a four-plus Wassermann and Kahn test. No characteristic clinical signs of syphilis could be found, and the sputum was negative for spirochetes by dark-field examination. Clinical syphilis of the lungs is reported to be very rare by practically all observers, and with the finding of monilia in the sputum it seems reasonable to exclude syphilis as being responsible for the lung lesions.

The nodular lesions of the face were differentiated from leprosy and *Tuberculosis cutis.* Nodular lesions of leprosy as a rule are easily diagnosed by the finding of numerous acid-fast bacilli, many of which are characteristically arranged in globi. The failure to find these organisms at once should put the observer on guard for some other etiologic agent. To rule out *Tuberculosis cutis* it was necessary to find another etiologic agent as the finding of tubercle bacilli in these lesions is sometimes very difficult. The observing of the yeastlike organism in smear and the histologic picture aided in this.

The subcutaneous tumors of the wrist and forearm could be diagnosed only after histologic section and culture with the finding of monilia.

The bone lesions of the fingers might well be looked upon as being caused by the same organism although no proof of this can be given. These lesions were closed except for one spot on one of the fingers, and it did not seem justifiable to disturb such a lesion for a specimen. However, bone lesions have been reported as being caused by yeastlike organisms. The absence of anesthesia seemed to rule out leprosy with which the bone absorption could have been associated.

It is interesting to note the finding of foreign body giant cells in the nodular lesion on the patient's face and in the lesion produced in the omentum of the white rat.

SUMMARY

A case of monilia infection of the skin and lungs with the production of subcutaneous myxomatous tumors and possibly the involvement of bone, in a colored man, is presented.

Bacteriologic studies showed that the organism was a strain of monilia.

Experimental data prove the organism is pathogenic for rabbits, white rats, guinea pigs, and mice in the order named. Inflammatory tumor masses were produced in the omentum of white rats, at the site of injection in guinea pigs and in the omentum of one mouse in which monilia were found to be present.

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BACTERIOPHAGE THERAPY IN BACILLARY DYSENTERY*

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INTRODUCTION

THE therapeutic value of bacteriophage in bacillary dysentery has not yet been established. Diversity of opinion as expressed in the literature may be due, in part, to the fact that some of the early reports were based upon treatment carried out in places where only meager laboratory facilities existed. These reports must be evaluated with due consideration for the three variables present in the clinical application of bacteriophage therapy to any infection: the virulence of the invader, its susceptibility to lysis by phage, and the quality of the phage employed with respect to potency and polyvalence. Failures have occurred in some instances possibly on account of delay in the use of adequate amounts of phage.

LITERATURE

D'Herelle,[1] in 1921 and Da Costa Cruz in 1923[2] and 1924[3] reported favorable results in treating Shiga dysentery with 2 c.c. of bacteriophage. No mention was made of in vitro susceptibility tests in either instance. In 1924 Spence and McKinley[4] treated Shiga and Flexner dysentery with a phage, isolated from a stool filtrate. Twenty patients were treated, nineteen within the first week of illness, with 10 c.c. of this phage given by mouth three times a day. The mortality rate was 10 per cent and the average period of hospitalization 5.8 days. A control group in another hospital, given no phage, had a mortality rate of 40 per cent, and an average period of hospitalization of 12.8 days. In Egypt, Compton,[5] in 1929, reported favorable results and a decreased death rate in treating 200 cases of bacillary dysentery. He used a phage obtained from d'Herelle together with three races isolated in Egypt, and gave a total dose of 6 c.c. Choudhury and Morison[6] in 1929 treated 80 cases of Flexner and Shiga dysentery with a polyvalent bacteriophage, giving 2 c.c. three times a day the first day, and twice a day thereafter. Because of limited laboratory facilities, no susceptibility tests were made. The mortality rate was only 4 per cent.

In contrast to these apparently favorable results, Davison[7] in 1922 reported a mortality of 58 per cent in a group of 12 children treated with bacteriophage. Dysentery bacilli (Flexner type) were isolated from the stools of 10 of these patients. Of the 8 strains tested in vitro, 7 were susceptible to the phage used. Comparatively large and frequent doses were given, the total dose ranging from 5 c.c. to 1,381 c.c. Seven patients were treated with phage orally and 5 by enema. Failure in this instance was attributed to the

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fact that therapy was started too late in the course of the disease. Taylor, Greval and Thant,[8] in 1930, reported bacteriophage treatment of 14 Shiga and 6 Flexner dysentery cases in which only a short interval elapsed between onset of the disease and treatment. Two cubic centimeters of polyvalent phage were given three times a day. In the treated Shiga group, there was a mortality of 12 per cent and in the control group, 14 per cent. Out of 6 patients in the control and treated Flexner groups, there was one death in each series. Riding,[9] in 1930, reported 60 cases, 30 patients treated with phage by mouth and 30 untreated. Six types of dysentery bacilli were isolated, and all but 12 strains were susceptible to the phage used. No clinical benefit was noted in the phage-treated patients. In 1932 McCay[10] reported treating a large number of patients in Calcutta with bacteriophage. Dysentery bacilli of the Flexner type were isolated in most of the cases. Twenty-five cubic centimeter doses were given twice a day, and in more severe cases this dose was increased. The deaths in the phage-treated group were 5.4 per cent, in the control group 10.8 per cent. The average time of hospitalization in the treated group was more prolonged than in the control group, although 30 per cent of the phage-treated patients recovered rapidly. In spite of rather conflicting evidence, McCay concludes that this form of therapy was not generally beneficial. In 1933, Johnston, Ebbs and Kaake[11] found that bacteriophage did not affect the clinical course of the dysentery. Of the 70 patients considered, all infants under two years, pathogenic organisms were isolated from the stools of 73 per cent. Only 17 strains of the 94 tested in vitro were lysed by the bacteriophage employed. The dosage used was one ounce every hour. Kessel and Rose,[12] in 1933, reported 68 cases, half of which were treated, the remainder serving as controls. Ninety per cent of the Flexner strains were found susceptible to the phage employed. The dosage was 3 c.c. to 5 c.c. of phage by mouth every twelve hours. There were 3 deaths in the control group and 4 deaths in the treated group. The period of hospitalization was somewhat shorter in the treated group.

EXPERIMENTAL

During the summer of 1934, an epidemic* of bacillary dysentery developed in Jersey City, New Jersey. Stool specimens from several of the patients were sent to our laboratory for diagnosis and bacteriophage susceptibility tests. From these specimens dysentery bacilli (Flexner group) were isolated. This organism was found to be completely susceptible, holding its lysis for twenty-four hours when treated with a mixture of approximately forty races of bacteriophage active against the colon bacillus.

With the dysentery strains isolated from these specimens, we prepared bacteriophage in beef infusion broth which was used extensively in Jersey City and the surrounding territory.

Although there were, during the epidemic, approximately 200 cases of bacillary dysentery treated with bacteriophage supplied by this laboratory, difficulty was encountered in evaluating the results, due to the fact that in the majority of cases the records were not made available to us. Through the

*A preliminary report upon this epidemic has been made by Felsen, Rundlett, Sullivan and Gorenberg[13].

courtesy of two of the hospitals and of the Hudson County Board of Health, records were obtained from which some generalization can be made. These cases may be divided into groups as shown in Table I.

TABLE I

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Number of patients	9	7	5	1
Phage therapy begun after admission to hospital	First day	2-4 days	5-15 days	Control
Average time for decrease of stool frequency	4⅔ days	7 days	12 days	12 days
Average days of hospitalization	18	13	28	22

Group 1 (9 patients) received phage on the day of admission; four and two-thirds days elapsed before the stools decreased in number and eighteen days of hospitalization were required. Group 2 (7 patients) received phage between the second and fourth days after admission; stools decreased in number after seven days, and the patients remained in the hospital thirteen days on the average. Group 3 (5 patients) did not receive phage therapy until between the fifth and fifteenth day after admission, and these showed reduction in number of stools at twelve days as did the control case, and required the longest period of hospitalization (twenty-eight days).

Bacteriophage was administered in doses of 2.5 c.c. to 15 c.c. a day, the average dose being approximately 10 c.c. daily. In this group of 17 children and 5 adults, the children were more severely ill, but there were no fatalities. It was unfortunate that there was but one control case to report. However, it is evident that, in those patients receiving early treatment, the frequency of stools was lessened and the period of hospitalization was somewhat shortened.

During this epidemic we had the opportunity to observe several cases in this hospital under bacteriophage treatment. Dysentery bacilli (Flexner group) were isolated from the stools of these patients. The organisms were completely susceptible to lysis and a phage was made specific for these strains by serial passage. The preparation used in therapy for these hospital cases consisted of 50 per cent specific Flexner phage and 50 per cent of the stock phage. A detailed account of these cases follows:

CASE 1.—S. B., male, aged four years, one of a family of four children, was admitted to the hospital Aug. 10, 1934. He was severely ill, dehydrated, and had a temperature of 102° F., with a pulse rate of 120. During the preceding week his temperature had ranged between 101° and 103° F. He had been passing daily as many as fifteen light green stools containing blood and mucus, with vomiting several times a day. This child and his brothers had been playing with three neighborhood children who had previously had similar attacks of diarrhea with blood in their stools. On the day of admission and on each of the three days following, the patient was given infusions of glucose. On the first day he passed four yellowish green watery stools containing mucus. One of these specimens was found positive for dysentery bacilli (Flexner group) which were completely lysed by the bacteriophage. On the second day, bacteriophage therapy was begun. The patient received three 5 c.c. doses by mouth and a retention enema of 10 c.c. of phage diluted with 1 or 2 ounces of starch solution. Nineteen stools were passed on this day. Twenty-six hours after starting bacteriophage the stools became slightly more formed with less mucus and no blood. On the fourth day (August 13), the child passed 31 stools with less mucus than previously had been observed. The oral administration of bacteriophage was increased to four doses of 5 c.c. each. On the

fifth day the stools appeared more formed; bacteriophage was increased to five doses. On the seventh day the patient seemed brighter. On the ninth day the bacteriophage enemas were discontinued because of the inability of the patient to retain them. Convalescence was uneventful and the child was discharged from the hospital September 4.

CASE 2.—A. B., male, aged six years, was admitted to the hospital Aug. 11, 1934, eighteen hours after his brother S. B. The child presented less toxic symptoms than his brother and his temperature was lower at the time of admission. During the preceding twelve hours, the patient had had a temperature of 103° F. and had passed three loose, green, watery stools containing large amounts of mucus and blood. On the day of admission his temperature was 103.6° F., and pulse 112. A stool specimen sent to the laboratory was positive for

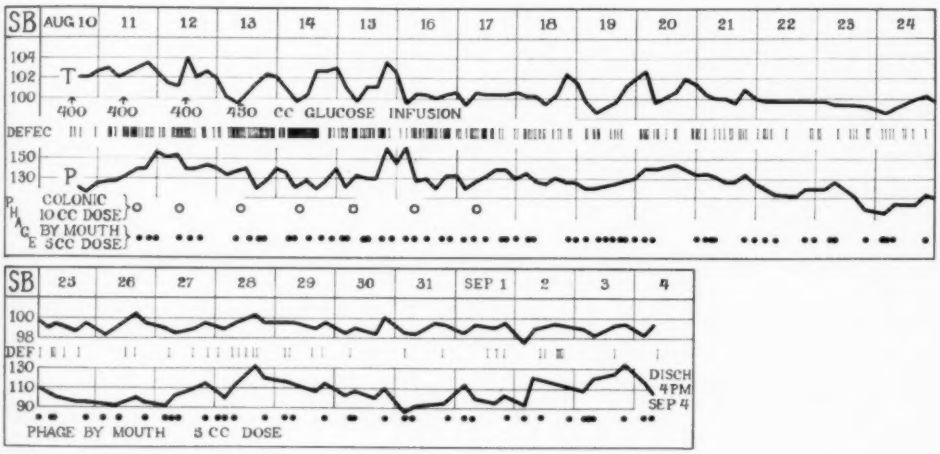

Chart 1.

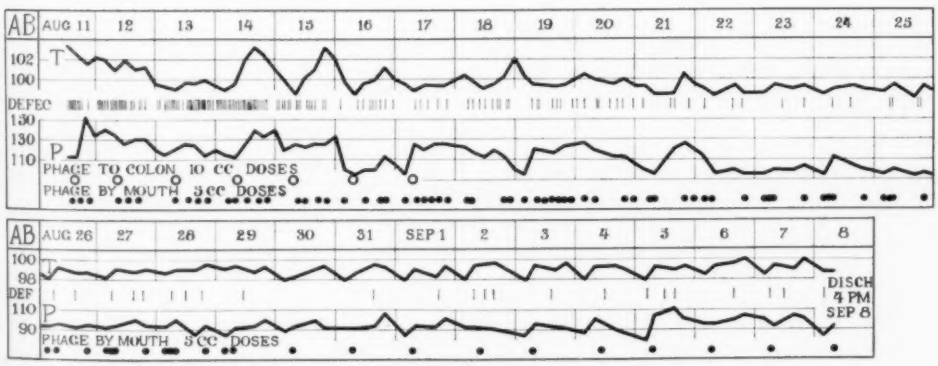

Chart 2.

dysentery bacilli. Bacteriophage therapy was begun at once with three doses of 5 c.c. each by mouth and a starch enema containing 10 c.c. of phage. The child passed twelve stools. On the second day, a stool passed in the morning was semiformed and contained less blood. On the third day his temperature varied between 99° and 100° F. and the child was more active. The stools were semisolid with less blood and mucus. Bacteriophage was increased to four doses of 5 c.c. each by mouth. On the fifth day, the stools were greatly decreased in number. The child appeared less irritable. On the thirteenth day (August 23), the stools were formed and there was no blood present. The patient was discharged September 8.

CASE 3.—J. B., male, aged seventeen months, brother of the preceding patients, was admitted to the hospital August 13. On the previous day he had passed three green watery

stools containing blood and mucus. On the day of admission bacteriophage therapy was begun with two doses of 5 c.c. each by mouth. He passed three green and brown semisolid stools with a moderate amount of mucus. A stool cultured on this day was positive for dysentery bacilli. The next day bacteriophage was increased to five doses by mouth and one

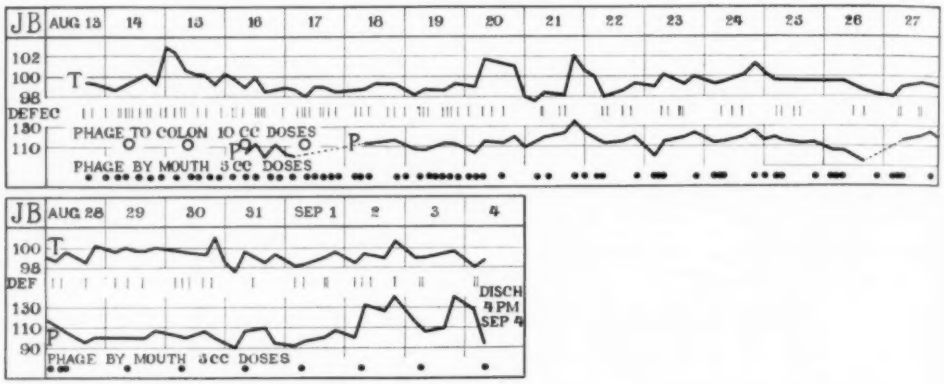

Chart 3.

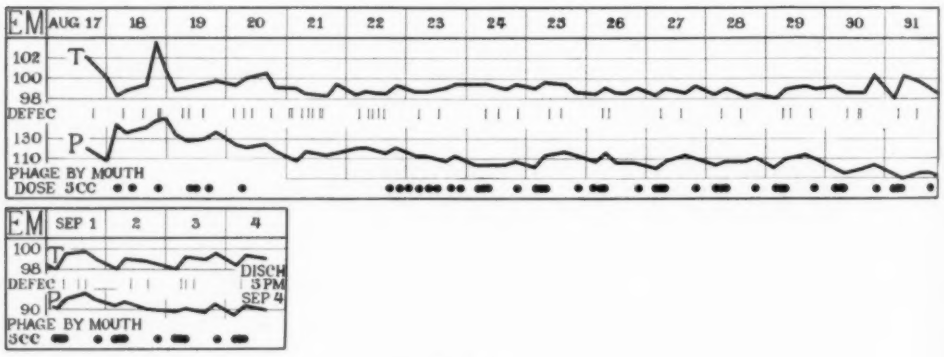

Chart 4.

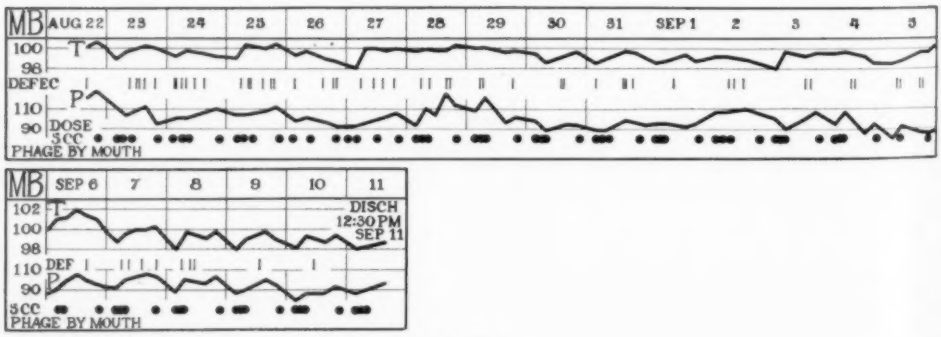

Chart 5.

10 c.c. enema was given. The patient passed ten stools, most of which contained blood. On the third day, he passed fewer stools and seemed much improved. On the tenth day, the stools were semisolid without blood. The patient was discharged September 4.

CASE 4.—E. M., male, aged twenty months, was admitted to the hospital on Aug. 17, 1934, with a temperature of 102° F. and a pulse of 120, fretful and severely ill. There was a

history of vomiting and diarrhea for five days before admission. Green watery stools with blood and mucus appeared the day preceding admission. The following day three 5 c.c. doses of bacteriophage were given by mouth. In the evening the temperature was 103.6° F. and the pulse 140. Four stools were passed this day, one of which was cultured and found positive for dysentery bacilli. The temperature decreased to 99° F. on the third day and he appeared less fretful. On the eighth day, the child passed three brown semisolid stools. He was discharged September 4.

CASE 5.—M. B., female, aged three years, was admitted to the hospital Aug. 22, 1934. During the six days preceding admission, the patient had passed daily from eight to ten loose, greenish brown stools which contained mucus and blood. For two days prior to admission, the child's temperature reached 102° F. On the day of admission, the temperature was 101° F. and pulse 120. One dose of 5 c.c. of bacteriophage was given. A stool specimen was positive for dysentery bacilli. The following day bacteriophage was increased to four doses of 5 c.c. each by mouth. On this day, five watery brown stools containing mucus and blood were passed. On August 26, the fifth day, four stools were passed which were free from blood although some mucus was still present. On the eleventh day, only one stool was passed and that appeared normal. The patient was discharged September 11.

DISCUSSION

The value of bacteriophage as a therapeutic agent in bacillary dysentery has not been fully determined. The future use of such therapy may be guided by an analysis of previous work, with due regard to the causes of success and failure. Coordination of the laboratory and the bedside will be required for a just appraisal of this newer form of therapy.

Although there is no standard dosage for the treatment of bacillary dysentery, it is probable that the bacteriophage should be given every day for a considerable period. We would stress the importance of starting bacteriophage treatment as early as possible after onset of the disease and employing larger doses than were used by the earlier workers.

For the successful use of phage in dysentery, it is important to determine the type of invading organism and to make in vitro tests for susceptibility of the strain to lysis. We prefer to use a strain-specific phage which has been adapted to the patient's strain of dysentery bacilli, by serial passage. We believe this kind of phage to be more efficacious than stock preparations.

SUMMARY

1. Detailed observations are presented upon five cases of bacillary dysentery in which specific bacteriophage was used in therapy.

2. A larger number of patients supplied with phage from our laboratory during a mild epidemic of dysentery in a neighboring community, provide a group from which general deductions may be drawn.

3. It seems advantageous to institute bacteriophage therapy as soon as possible after onset of the disease and to persist in its use.

4. The invading organism should be isolated and a strain-specific phage prepared for use without delay.

Acknowledgments.—We wish to express our appreciation of the helpful cooperation of the personnel of the Board of Health and Vital Statistics of Hudson County, New Jersey, Jersey City, New Jersey; of St. Mary Hospital, Hoboken, New Jersey and of St. Francis Hospital, Jersey City, New Jersey. We also desire to make grateful acknowledgement to Dr. Roger H. Dennett, deceased, on whose service the five hospitalized patients were treated.

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THE DIURETIC ACTION OF GLUCOPHYLLINE*

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J. W. L. Robinson, M.D., Washington, D. C.

GLUCOPHYLLINE, one of the newer diuretics of the xanthine series, is a double salt representing a mixture of theophylline and methyl glucamine, having a xanthine content of 38.7 per cent. It differs from aminophylline in that the latter represents a mixture of theophylline and ethylene diamine, having a xanthine content of 59 per cent.

In determining the diuretic activity of this newer preparation, we have carried on a series of experiments on laboratory animals and human volunteer subjects, the results of which are presented in this report.

LABORATORY EXPERIMENTS

Dogs were used for quantitative determination of the diuretic action of glucophylline. Both ureters were cannulated and the urine flow was measured for a specific time interval in each experiment. In most instances the blood pressure and respiration curves were also taken at the same time. In the first four experiments, the animals were anesthetized with dialurethane or nembutal, and the diuretic action of glucophylline was tested against that of citrated caffeine, aminophylline, and salyrgan. In the last ten experiments, to reduce complications, pernocton was used exclusively to produce anesthesia, and the

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diuretic action of glucophylline was tested against that of aminophylline only. In order to maintain a fairly constant condition of body fluid, slow hypodermoclysis or rectal drip with normal saline was given throughout the course of each experiment. No new drug was given until the urinary output under the preceding test drug had returned to the normal value for a given experiment.

The records show that in adequate doses the respiratory rate and volume were increased by all the diuretic agents. Of these, the mercurial diuretic salyrgan had the most pronounced effect. On the blood pressure an early but rather transitory fall was registered by all. Increase in urine excretion seemed to bear relation to restoration of the blood pressure to the preexisting level (or above), for in no instance did the optimal excretion occur until the blood pressure regained its normal level. There was observable, therefore, a definite latency with each compound. It was impossible, however, to construct comparative curves of latency because of the lack of uniformity of response on the part of the individual animals to the compounds tested.

An analysis of the results presented in Table I shows that glucophylline compares favorably in its diuretic efficiency with aminophylline. In the ten

TABLE I

DOGS: COMPARATIVE DIURETIC ACTION OF COMPOUNDS TESTED

DOG	WEIGHT KG.	ANESTHETIC	DRUG	DOSE MG./KG.	URINE EXCRETED
1	F. 6.5	Nembutal	Aminophylline	10.0	2.5
			Glucophylline	10.0	3.5
			Salyrgan	10.0	3.5
2	M. 15.0	Nembutal	Citrated caffeine	13.0	3.3
			Aminophylline	13.0	3.5
			Glucophylline	17.0	3.0
			Salyrgan	20.0	1.0
3	M. 13.5	Dialurethane	Citrated caffeine	10.0	2.3
			Aminophylline	10.0	1.0
			Glucophylline	10.0	3.0
4	M. 12.5	Dialurethane	Citrated caffeine	10.0	3.3
			Aminophylline	8.0	3.3
			Glucophylline	6.0	5.0
			Salyrgan	20.0	4.0
5	F. 4.6	Pernocton	Aminophylline	14.28	3.0
			Glucophylline	25.86	3.5
6	M. 14.2	Pernocton	Glucophylline	25.86	9.96
			Aminophylline	14.28	1.92
7	F. 8.2	Pernocton	Glucophylline	25.86	24.0
			Aminophylline	14.28	16.8
8	F. 8.4	Pernocton	Aminophylline	14.28	7.44
			Glucophylline	25.86	1.92
9	M. 13.4	Pernocton	Glucophylline	25.86	9.0
			Aminophylline	14.28	5.76
10	M. 13.6	Pernocton	Glucophylline	25.86	9.6
			Aminophylline	14.28	6.0
11	M. 5.7	Pernocton	Aminophylline	14.28	9.24
			Glucophylline	25.86	7.50
12	F. 5.6	Pernocton	Aminophylline	14.28	7.50
			Glucophylline	25.86	15.0
13	M. 13.4	Pernocton	Aminophylline	14.28	12.06
			Glucophylline	25.86	9.0
14	M. 13.6	Pernocton	Aminophylline	14.28	2.46
			Glucophylline	25.86	0.96

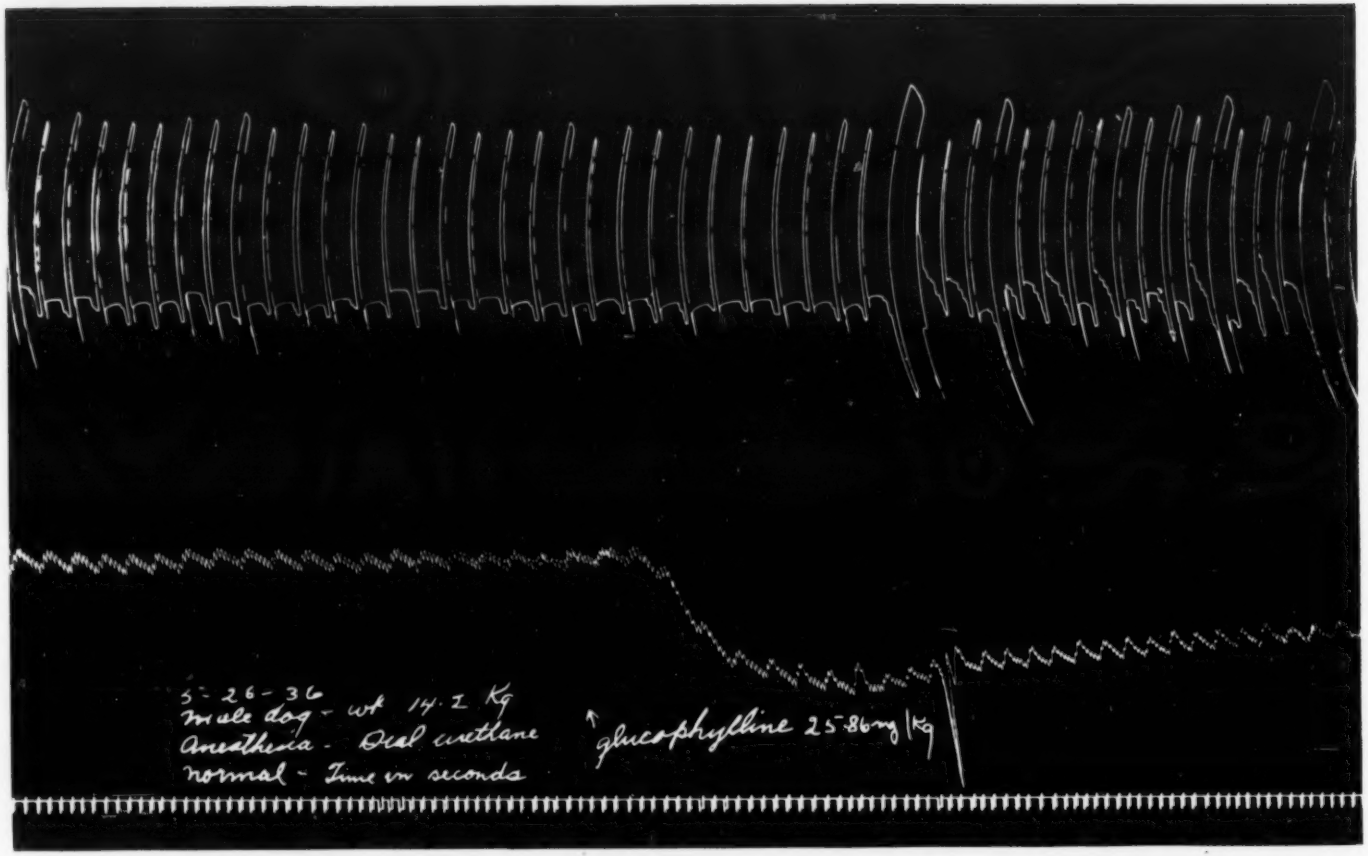

Fig. 1.—The effect of glucophylline on respiration and blood pressure; upper tracing, respiration; middle tracing, blood pressure; lower tracing, time in seconds.

experiments under pernocton anesthesia in which the doses of these two compounds represented an equivalency of xanthine content, the average hourly excretion was 9 c.c. for glucophylline as against 7.2 c.c. for aminophylline.

Typical tracings showing the effects of glucophylline and aminophylline on blood pressure and respiration are presented in Figs. 1 and 2.

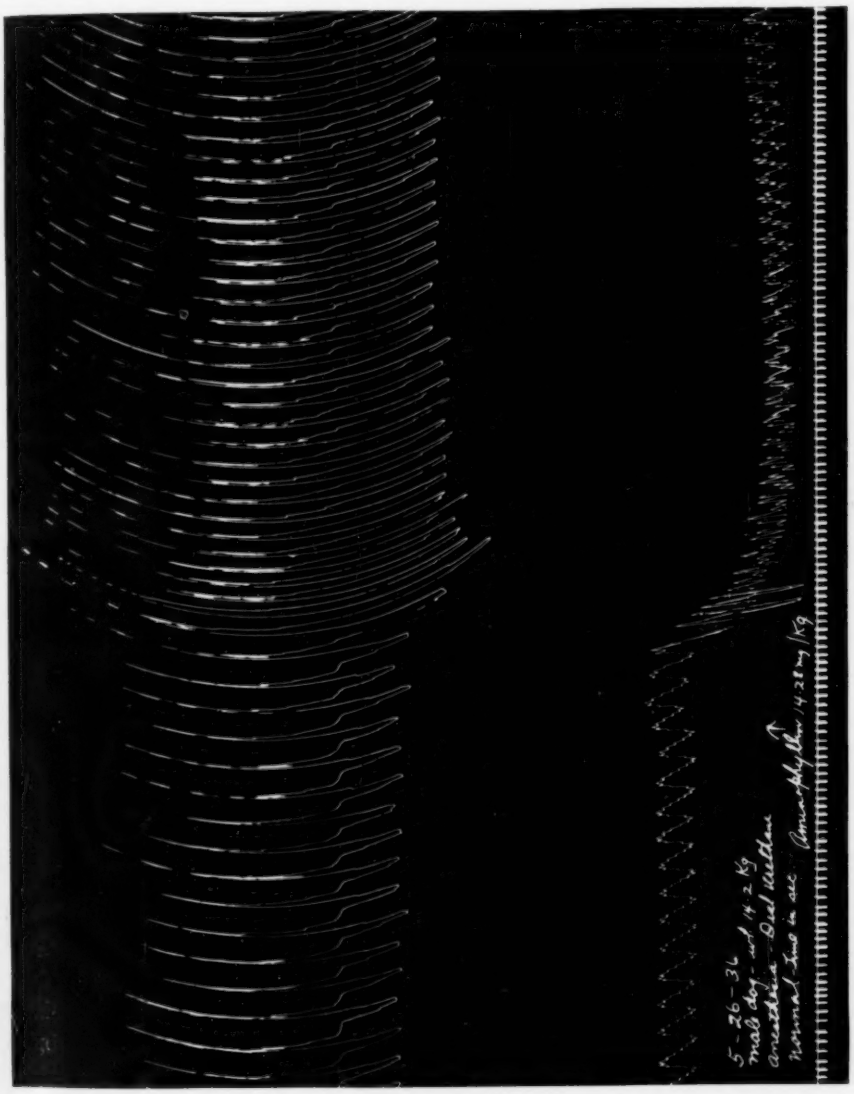

Fig. 2.—The effect of glucophylline on respiration and blood pressure; upper tracing, respiration; middle tracing, blood pressure; lower tracing, time in seconds.

CLINICAL EXPERIMENTS

Seven male medical students and two of us volunteered to serve as subjects for this group of experiments. A renal function test and a complete urinalysis were done on each subject as a preliminary step. All subjects were found to be normal as to functional capacity and their urines were negative for albumin and reducing substances. Four xanthine diuretics were employed for purposes of critical comparison with glucophylline: Theophylline sodium

acetate (theocin), theobromine sodium salicylate (diuretin), theophylline ethylene diamine (aminophylline), and caffeine alkaloid. In addition, a few series were run with methyl glucamine to determine the rôle played by this salt in the mixture. In no case was a subject informed what the drug was at the time of administration and, to further negative the psychic factor a series was run with bicarbonate of soda as the test drug. Our procedure was as follows: An average volume output of urine voided on six consecutive days was taken as the standard for comparative purposes. At 9:00 A.M. the urine was voided and discarded. All other samples for the day were kept, the last being at 5:00 P.M. This served as control. On test days the drug was given by mouth immediately after the 9:00 A.M. voiding and repeated at 12:00 M. Since it was practically impossible to secure a twenty-four-hour specimen, the duration

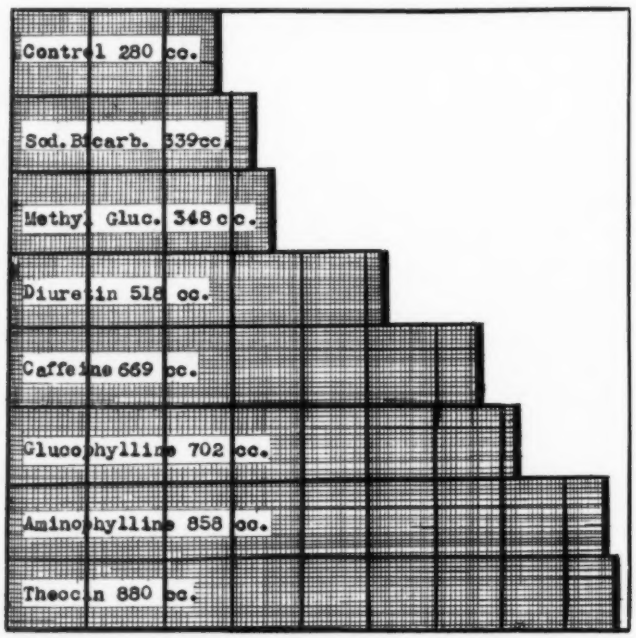

Fig. 3.—Composite averages on nine human subjects.

of action was determined by routine collections the following day. No restrictions as to diet or fluid intake were imposed, but to compensate for incidental variations more than one trial was made with each of the drugs and several with glucophylline. Routine urinalyses showed, in some, variation in the specific gravity and reaction and, in all, the constant absence of albumin and reducing substances. A record was kept on the volume output in each case. The dose of each compound was computed on the basis of its xanthine content, using caffeine as unity and bringing up the others to relative equivalency. Seven days were allowed to intervene between any two trials. Table II gives details on individual results.

Although this study is but roughly quantitative, certain definite deductions can be drawn from the figures presented in this table. Taking each of the nine subjects separately, glucophylline comes among the first three most effective diuretics, the other two being theocin and aminophylline, all belonging

TABLE II

HUMAN SUBJECTS: COMPARATIVE DIURETIC ACTION OF COMPOUNDS TESTED

SUBJECTS	1		2		3		4		5		6		7		8		9	
COMPOSITE NORMAL VOLUME, C.C.	220		380		330		250		315		280		195		380		270	
TEST DRUGS	VOL.	%	VOL.	%	VOL.	%	VOL.	%	VOL.	%	VOL.	%	VOL.	%	VOL.	%	VOL.	%
Glucophylline	927	421	643.3	70.0	920	280	525	210	709	225	538	192	473	243	930	245	1560	578
Theocin	1155	725	645.0	67.1	1365	413	698	279	893	283	575	205	607	311	945	249	1280	475
Aminophylline	1410	641	437.5	15.0	1205	365	528	211	820	260	693	248	785	403	1200	313	775	287
Diuretin	908	412	492.5	40.0	438	133	325	130	763	244	313	223	338	173	480	126	410	152
Caffeine	870	395	640.0	168.5	455	138	650	260	908	288	615	219	605	310	540	142	685	254
Methyl glucamine	375	170	327.0	86.0	305	93	220	88	360	114	368	131	262	134	455	120	890	330
Sodium bicarbonate	450	204	290.0	76.0	510	155	270	108	290	92	240	86	320	164	–	–	–	–

to the theophylline group. This is in good agreement with the general observation that the theophylline group is more powerfully diuretic than the caffeine or the theobromine group. A determination of the diuretic effects on the second day showed that glucophylline has the longest duration of action. This is probably due to the methyl glucamine fraction for our protocols show this substance to be more active on the second even than on the first day. In chronic conditions, glucophylline possesses this advantage over aminophylline especially, which exhibits what appears to be a compensatory oliguria on the second day. In almost all instances theocin and glucophylline produced a change toward alkalinity in the reaction of the urine. This stands out in striking contrast to the other diuretics under investigation which almost uniformly produced an acid or neutral reaction. There is close correlation also in composite average figures on the diuretic efficiency of the various substances. Reference to the composite averages in Fig. 3 shows that glucophylline stands third in the series.

DISCUSSION

In both anesthetized dogs and normal human beings, glucophylline acted as a good diuretic agent. In our experiments, it exhibited an action superior to caffeine but similar to the other salts of theophylline. The only observable difference noted with respect to theocin and aminophylline was its longer latency. Its slightly reduced efficiency would be clinically inconsequential. It should be of interest to determine whether glucophylline exerts any untoward effects on the liver or kidney. Accordingly a series of chronic animal experiments are in progress with the view of studying these organs and other viscera histologically after a reasonably extended period of frequent medication.

SUMMARY

The results obtained on laboratory and clinical investigations of the diuretic action of glucophylline, the double salt of theophylline and methyl glucamine are presented.

The laboratory studies were made on dogs.

It was found that the average hourly urine excretion under glucophylline amounted to 9 c.c.; that under aminophylline to 7.2 c.c.

The clinical studies were made on seven sophomore medical students and two instructors.

In each individual case, glucophylline compared favorably with aminophylline and theocin and was superior to caffeine and diuretin.

In the composite averages of the nine subjects, glucophylline ranked third in the list of diuretics employed for comparative investigation. The diuretic action of theocin was 880 c.c., aminophylline 858 c.c., glucophylline 702 c.c., caffeine 669 c.c., and diuretin 518 c.c.

Glucophylline has a longer duration of diuretic action than aminophylline. This is probably due to the methyl glucamine fraction, which as a diuretic is more active on the second than the first day.

Appropriate tables and figures are presented.

We wish to thank the following pharmaceutical houses for supplying the drugs used in this work: The Abbott Laboratories for glucophylline and methyl glucamine, the H. E. Dubin Laboratories, G. D. Searle & Co., for aminophylline, Hoffmann-La Roche, Inc., for diuretin, and the Winthrop Chemical Co., for theocin.

HEMOGLOBIN STUDIES ON COLLEGE WOMEN WITH SPECIAL REFERENCE TO THE EFFECT OF MENSTRUATION*

DOROTHY DUCKLES, AND C. A. ELVEHJEM, MADISON, WIS.

THIS study of the hemoglobin content of the blood of seven college women, ranging from twenty to twenty-seven years of age, was undertaken to determine the daily and hourly fluctuations which may occur. Hemoglobin determinations were made for a period of fifty-two consecutive days, covering approximately two menstrual cycles. One complete cycle represented for each woman was divided into the four phases: premenstrual, menstrual, postmenstrual, and intermenstrual; and an effort was made to correlate hemoglobin changes with these various phases. Hourly hemoglobin determinations were made on three of the subjects, once during the intermenstrual period and also on the second day of menstruation, to study the changes occurring within the day.

LITERATURE

A survey of the literature indicates that this subject is one which has not been studied extensively but has received increased attention during the past few years. Williamson,[1] in 1916, investigated the influence of age and sex on hemoglobin and reported that from the ages of sixteen to fifty-five years the variations were slight. During that period of life, the values for women were found to be appreciably lower than for men: 15.53 gm. per 100 c.c. as compared to 16.92 gm. More recent work has shown that this standard for women, particularly, is probably too high. In 1927, Lippincott[2] concluded that 13.7 gm. was the average hemoglobin for females. Murphy and others[3] reported an average of 13.9 gm., and Helmer and Emerson[4] gave a similar figure of 13.68 gm. Sachs and coworkers[5] have suggested a slightly lower value of 12.96 gm. for women, in comparison to 14.96 gm. for men. From these data, a range of 13 to 14 gm. of hemoglobin per 100 c.c. of blood would seem to be normal for women.

That diurnal variations in the hemoglobin content of the blood are marked has been realized but not given serious attention. In 1920 Dreyer and associates[6] noted that these fluctuations were as high as 30 per cent and that a change of 10 per cent was a common occurrence. They gave as possible causes of these fluctuations: blood pressure, rate and volume of respiration, variations in pulse rate, and possibly fluid absorption and kidney excretion.

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In a recent study reported by Leverton and Roberts,[7] they found that the standard daily deviation for women was 0.9 gm. of hemoglobin per 100 c.c., even in subjects under controlled conditions.

Rabinovitch,[8] in 1923, studied the hourly hemoglobin changes in 20 normal male individuals from 8 A.M. to 6 P.M. As much as 26 per cent variation was found in 2 cases, in 4 cases the variation ranged from 15 to 20 per cent, and in 6 cases from 10 to 15 per cent. Mills[9] investigated the hourly changes which occurred in persons with secondary anemia and found only a 4 per cent fluctuation. He concluded that the hourly irregularities were less pronounced in anemic persons than in normal healthy persons. On the contrary, Dreyer and others[6] found a more marked daily deviation in persons with a low hemoglobin level.

It does seem to be generally conceded that there are both daily and hourly fluctuations in hemoglobin and that these are more pronounced in women than in men. Ingersoll[10] found a 1.54 gm. average difference in women as compared to 1.10 gm. in men. Whether or not the variation in females can be attributed, at least, partially to menstrual changes causing an instability of the hematopoietic system and increased physiologic demands for iron is a subject of controversy. Smith, 1936,[11] studied 16 menstrual periods and concluded that increases and decreases in hemoglobin during the menstrual period were no different than during the intermenstrual period. Leverton and Roberts[7] summarized their data, after making an extensive study, by stating that the effect of menstruation was not definite or consistent. Reich and Green[12] pointed out that there was no postmenstrual reticulocyte peak, which would be evidence that there is no definite blood regeneration following menstruation. In contradiction to these findings, Ashby[13] reported that the blood-destroying activity in women was coincident with menstruation. Rowe and Guagenty[14] found that there was a downward trend during the time of menstrual flow and a gradual recovery after its termination. However, the average change was not great.

Barer and associates[15] analyzed the normal menstrual loss for 50 healthy women, nineteen to forty-three years of age, who presented no known menstrual disorders. They found that 3.84 to 78.4 mg. of iron was lost per period, representing 1.146 to 23.403 gm. of hemoglobin. This loss was equivalent to 9.39 to 207.28 c.c. of blood, calculated from the individual's own hemoglobin content in grams per 100 c.c. Patients with hypochromic anemia, even though they considered their menstrual periods normal, showed larger losses. According to their calculations, a loss of 78.4 mg. of iron each menstrual period would require a daily iron retention of about 2.8 mg. to replace that lost.

EXPERIMENTATION

Seven healthy college women, twenty to twenty-seven years of age, served as subjects for this investigation. Five of them, Cases 1 to 5, had had hemoglobins taken at intervals before the study was begun and were known to be nonanemic individuals. The sixth woman, Case 6, had shown a rather low hemoglobin level for a short period about six months previous to this time. She was given iron therapy, 25 mg. daily in the form of ferric pyrophosphate,

for three weeks, and her hemoglobin was raised from 12.40 to 13.89 gm. per 100 c.c. of blood. At the time this experimental work was undertaken, the same treatment was started again, although her hemoglobin was 13.61 gm. She continued taking iron for the fifty-two days during this investigation. The seventh subject, Case 7, had been a known diabetic for thirteen years. She was taking 30 units of insulin daily and was receiving a carefully weighed adequate diet. She was in good health but had an average hemoglobin of approximately 12 gm. per 100 c.c. of blood, so was given iron and copper throughout the fifty-two-day period. Twenty-five milligrams of iron in the form of ferric pyrophosphate and 1 mg. of copper from copper sulphate were administered daily. She had been receiving the same therapy at intervals

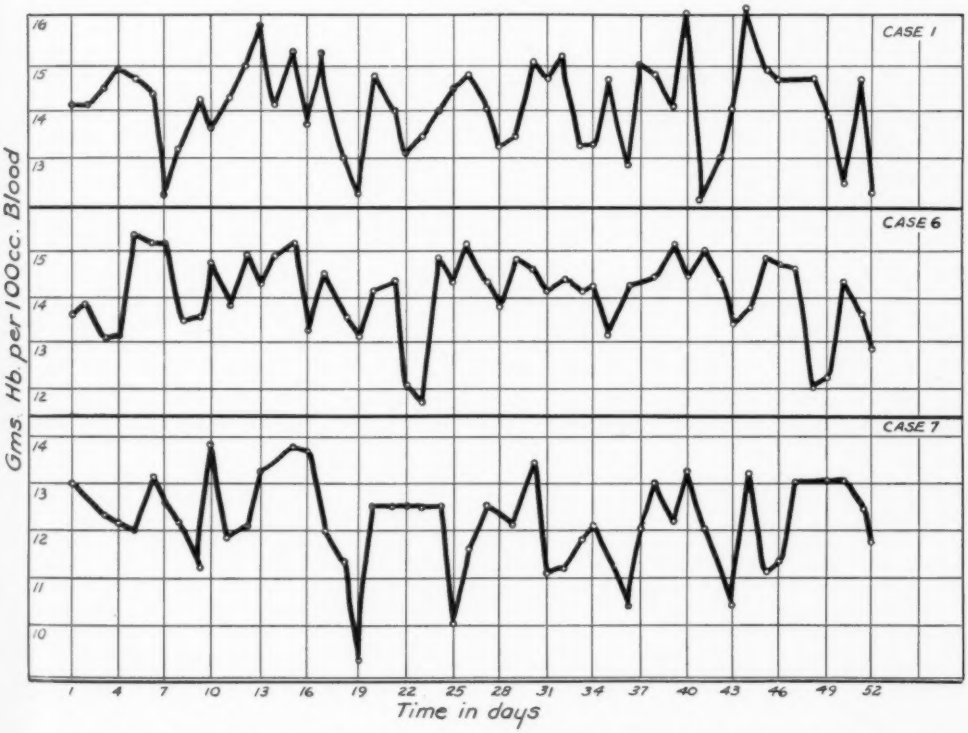

Chart 1.—Typical individual curves showing daily variations in the hemoglobin content of the blood. Case 1 normal woman, Case 6 receiving 25 mg. Fe daily, Case 7 a diabetic receiving 25 mg. Fe and 1 mg. Cu daily.

previous to this time. All of the women were home economics students at this university, had studied dietetics, and were familiar with the requirements of an adequate diet. Their nutrition was good.

Hemoglobin determinations were made daily with few exceptions for a period of fifty-two consecutive days in order to observe the variations which occur. Cutaneous blood samples were taken from the fingertips. Each person's hemoglobin was taken at a corresponding time each day, usually from 10 A.M. to 1:30 P.M. The Newcomer method was used to determine the hemoglobin, the sample being compared with a carefully standardized disc in a Bausch and Lomb colorimeter.

Chart 1 shows a typical individual graph for one of the 5 subjects who had no history of anemia and also gives the records of the 2 women who were receiving treatment for slight hypochromic anemia. It will be noticed that the patient in Case 6 has a hemoglobin within the normal range, averaging 14.13 gm., throughout the study; while the patient in Case 7 shows a hemoglobin level lower than the others, averaging 12.19 gm., although she was receiving treatment. The daily variations are significant in all cases. They do not appear to be any more or less marked in Case 7 than in any of the others.

Chart 2 summarizes the average hemoglobin values computed from 214 determinations on the 5 women whose hemoglobins were consistently nonanemic. The range is from 13 to 15 gm. of hemoglobin per 100 c.c., and the average is 14.29 gm. Although this figure is based on a study of only 5 individuals, it probably represents a true average of the standard hemoglobin value for women of this age, since determinations were made almost daily for fifty-two consecutive days on subjects who were known to be normal healthy women. Certainly, a single hemoglobin determination is not likely to be

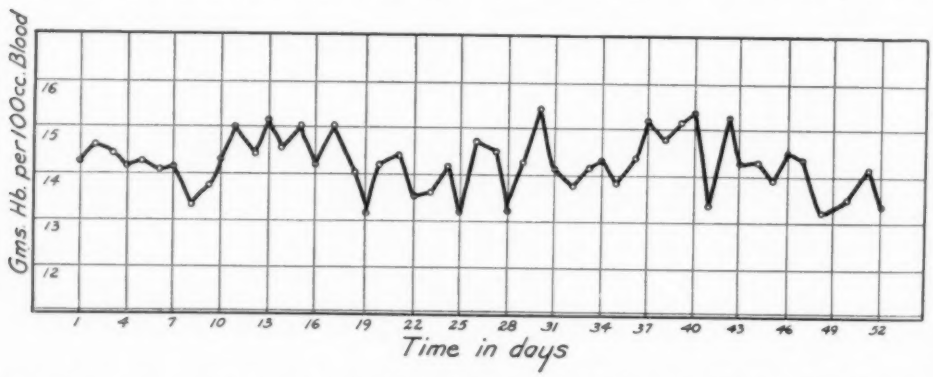

Chart 2.—Average hemoglobin curve constructed from 214 determinations on 5 normal cases.

indicative of the average level. Since determinations are often made by methods far less accurate than the colorimetric one used in this study, there is striking evidence as to the limitations of making a clinical diagnosis on the basis of one or two readings.

Hourly determinations were made on the patients in Cases 1, 3, and 6 to study the changes occurring within the day. The blood samples were taken from 7 A.M. through 10 P.M., and the colorimetric readings were made by daylight the following morning. Two one-day periods were taken as probably being exemplary of the hourly changes which usually occur. One day was during the intermenstrual period, approximately midway between menstrual periods, and the other was the second day of menstruation. This is the day of greatest hemorrhage for most healthy women.[16] Each subject had eight hours' rest the night before the hourly readings were made, and a careful record of food consumption was kept. The days chosen were typical days of activity.

In Chart 3, the two daily records for each of the 3 women are presented. In all cases, the irregularities are appreciable. The variations on the second

day of menstruation are no more marked than those occurring during the intermenstrual period. There seems to be a slight downward trend in hemoglobin during the day. This is particularly true in Case 1, where the hemoglobin falls from 15.43 gm. in the morning to 12.63 gm. in the late evening during the midmenstrual period, and from 16.23 gm. to 13.89 during the menstrual period. Although this small amount of data is inadequate, there is no correlation between the 3 cases which would warrant concluding that the menstrual loss affects the hourly hemoglobin deviation.

According to Dodds,[17] the complete menstrual cycle can be divided into four phases: (1) premenstrual period of four or five days, during which there is an increased congestion of the mucosa of the uterus; (2) menstrual period

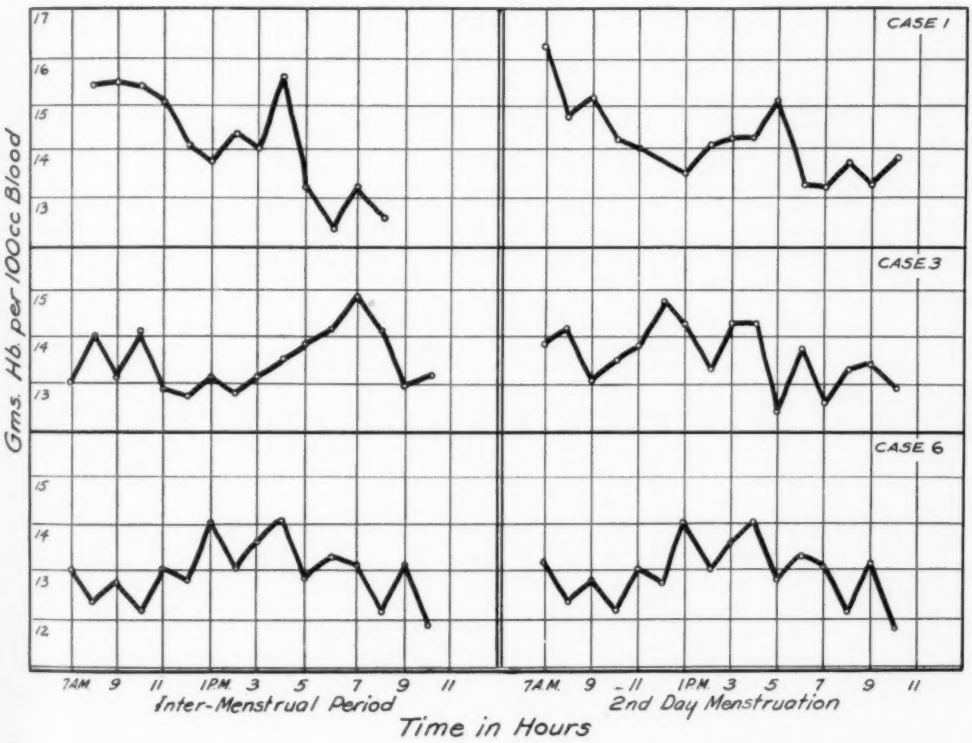

Chart 3.—Curves for 3 cases showing hourly variations in hemoglobin.

usually of four or five days marked by hemorrhage from the uterine mucosa and some destruction of its tissues; (3) postmenstrual period of about seven days during which the uterine mucosa returns to its original condition; and (4) intermenstrual period during which the uterus is at rest. Chart 4 shows individual graphs presenting the average hemoglobin during the four different phases of the menstrual cycle for the seven college women studied. Five days were chosen as representative of the premenstrual period and seven days of the postmenstrual period. The menstrual periods varied with the different individuals, ranging from three to five days. The total cycles varied from twenty to thirty-three days. In Cases 1, 2, 3, 5, and 7, the lowest hemoglobin value is reached during the menstrual period. In some instances, there is a

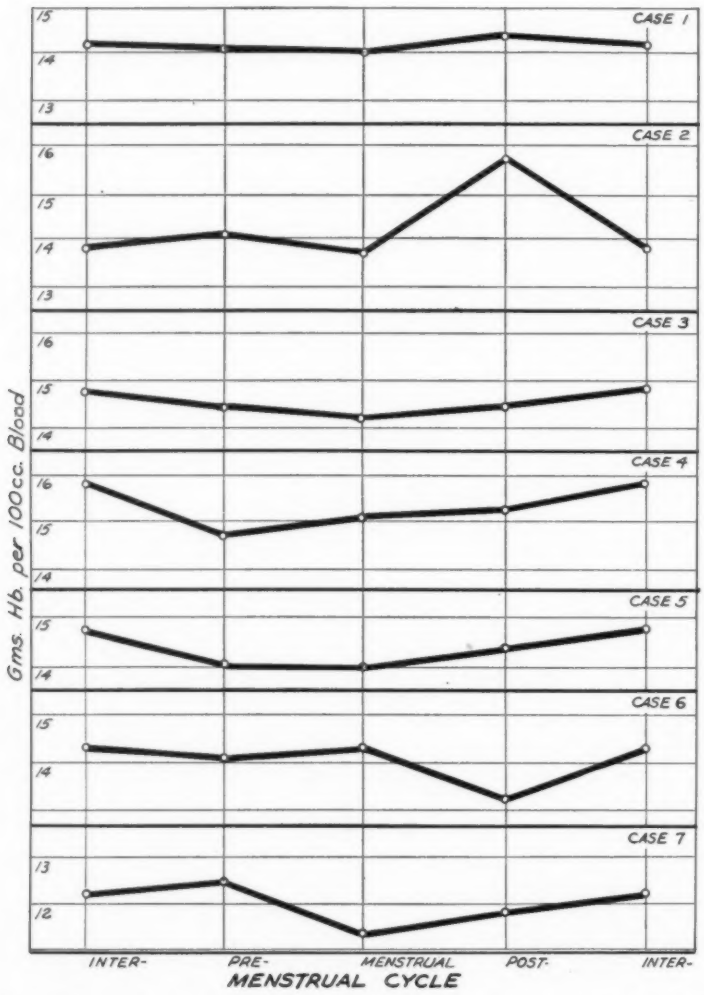

Chart 4.—Individual curves for 7 cases showing average hemoglobin values during the 4 phases of the menstrual cycle.

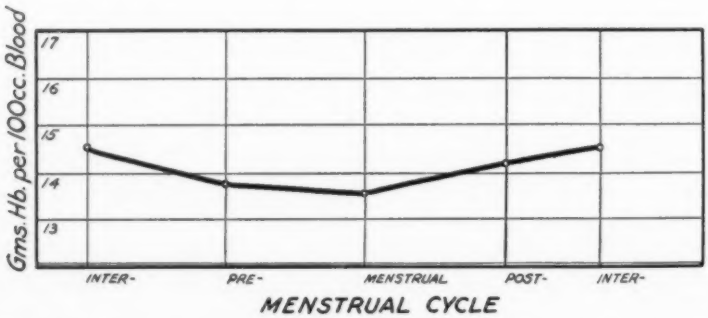

Chart 5.—Average curve constructed from 7 cases showing hemoglobin change with menstruation.

much more pronounced drop than in others. This can be explained easily when we consider that the menstrual loss may vary from 1.146 gm. to 23.403 gm. of hemoglobin for healthy women.[15] Also, when it is realized that hourly fluctuations occur which are not consistently the same at corresponding hours on different days, it is not surprising that two of the cases show a slight rise in hemoglobin for the average during the few days of menstruation. It is interesting to note that the one individual studied who was definitely anemic shows the greatest drop in hemoglobin during the menstrual phase of the cycle, though she considered her menstrual periods normal. Barer and associates[15] found much greater menstrual losses in patients with hypochromic anemia.

In Chart 5 averages are made of the seven individual graphs presented in Chart 4. It shows that there is a peak of 14.55 gm. of hemoglobin per 100 c.c. of blood during the intermenstrual phase of the cycle and that the lowest ebb of 13.61 gm. is reached during the menstrual period, which is characterized by hemorrhage. During the postmenstrual phase, there is a rise in hemoglobin up to 14.20 gm. which continues to increase slightly during the next intermenstrual phase. Although the average difference between the intermenstrual and menstrual phases is only 0.94 gm. in the seven cases studied, this diminution may be considered significant.

SUMMARY

1. The average hemoglobin for healthy women, twenty to twenty-seven years of age, is approximately 14 gm. per 100 c.c. of blood and shows daily variations ranging from 13 to 15 gm.

2. Hourly fluctuations in hemoglobin occur which are not consistently comparable at corresponding hours in different days. The hourly variations are as marked during the intermenstrual period as during the menstrual period.

3. There is usually a diminution in the hemoglobin during menstruation, the amount varying considerably with different individuals. During the postmenstrual phase of the cycle, there tends to be a rise in hemoglobin which continues to increase slightly during the intermenstrual phase.

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THE KAHN TEST IN MALARIA*

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THE first communications[20] regarding a positive Wassermann reaction in malaria date back to the years 1908 and 1909 when Michaelis and Lesser, Much and Eichelberg and others raised the question. These reports together with his own observations led A. von Wassermann, the originator of the test, to despatch G. Meyer to Italy for the purpose of studying the matter. Meyer reported that 80 per cent of all cases of malaria with parasites in the blood showed a positive Wassermann reaction. Since then most observers have confirmed these findings, though in varying degrees.

Craig, in Hagen's textbook[1] says: "Of the few conditions in which a positive Wassermann reaction sometimes occurs are . . . malarial fevers, during the febrile stage." During the past twenty years a large number of observers have reported their experience with the Wassermann test in malarial infection. Without attempting to exhaust the list, one may mention St. John[2] who found a positive Wassermann reaction in 4.9 per cent of his malarial patients; Curth,[3] in 12.5 per cent; DeHaan,[4] in 19 per cent; Mayr,[5] in 20 per cent; Schilling,[6] in 33 per cent; Hirsch,[7] in 36 per cent; Hebewerth and Kop,[8] in 50 per cent; Fischer and Günsberger,[9] in 75 per cent; Nagell,[10] in 84 per cent. All of them excluded patients that gave a history suggestive of syphilis and state that the reaction became negative in a period varying from several days to many weeks after defervescence.

The great variation in the frequency of these false positives as reported by the various observers can only be explained by differences in technic and justifies at least the suspicion that Kolmer[11] may be right when he asserts that the so-called false positives in malaria are always due to faulty technic. Using his own modification of the Wassermann test, he found that in 26 unselected cases of tertian and estivo-autumnal malaria none gave a positive Wassermann reaction. In another series of malarial serums, sent him from Panama and Texas, all were negative to the Wassermann test except 2 in whom the possibility of syphilis could not be excluded. Businco and Foltz[12] found a positive Wassermann reaction in 5 out of 49 cases of malaria but state that in all 5

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there was definite reason for suspecting the presence of syphilis. Johnson[13] studied the serums in 74 cases of malaria by means of four different modifications of the Wassermann test. His percentage of positive reactions varied from 6 to 27 per cent. Most of the cases that gave a positive reaction were still positive when retested some time after defervescence, though the period of observation was apparently not long. At least one of these was found later to be syphilitic, and Johnson thinks they all were. In 50 cases of malaria, Levy[14] found the Wassermann reaction positive four times. One of the positives gave a venereal history; another with a persistent positive reaction became negative after mercury salicylate injection. His impression is that "when a positive Wassermann reaction is obtained during a malarial infection, we have a syphilitic infection in addition." McConnell[15] reports a suggestive case. A patient with a fatal pernicious malaria showed a four-plus Wassermann reaction. There was no other reason for suspecting a syphilitic infection, but at autopsy a syphilitic mesaortitis was found. Saunders and Turner[16] made a careful study of 1,608 patients in a yaws clinic at Bath, Jamaica. Of these, 266 were listed as malarial, and these gave almost the same proportion of positive Wassermanns as the general population. In most of them the reaction remained positive, whether in an attack or not. In only two was the reaction positive during the malarial paroxysm and negative at other times; both of them, however, showed evidence of a previous infection with yaws. The authors suggest that in an old yaws patient, whose Wassermann has become negative, a malarial infection may again cause the latent Wassermann to become positive. Heinemann[17] had made a similar observation. He stated that an old syphilis or frambesia patient may have a positive Wassermann reaction made negative by malaria, to return to positive after the conclusion of treatment; while, on the other hand, a syphilitic with negative serology may have the Wassermann made permanently positive by a malarial infection.

One of our cases points a similar lesson. H. H., a paretic, had a four-plus Kahn in his blood serum in 1931 with a three-plus Kahn and a paretic gold curve in the spinal fluid. Fairly vigorous treatment resulted in negative serology without neurologic improvement. In July, 1934, he entered the hospital for malarial therapy. At the time, the blood Kahn was negative; the spinal fluid showed a negative Kahn but 74 cells per c.mm. The day after his first malarial paroxysm, the blood Kahn became three-plus, the Wassermann reaction three-plus. The following day both of the tests were four-plus, and have remained so ever since.

The most recent publication on the subject is that of Wilson and Levin,[21] who reviewed the material at a large general hospital in Charleston, S. C. Of 262 cases of malaria, 192 gave a negative and 70 a positive Wassermann. Of the 70 positive reactions, 13 were definitely not syphilitic as was shown not only by the history and physical findings, but also by the fact that in all of them the serology became negative after defervescence. These 13 false positive reactions represent about 5 per cent of the total number of malaria cases; about 4 per cent of the total number gave a falsely positive Wassermann reaction and less than 3 per cent a falsely positive Kahn reaction. As will be seen, these are much lower figures than we found in our own cases. Another in-

teresting observation was that, whereas only 2 of these showed a positive Kahn and a negative Wassermann reaction, 6 showed a negative Kahn reaction and a positive Wassermann reaction. In our series, no patient with a positive Wassermann reaction failed to give a positive Kahn test.

These findings leave one in something of an impasse. On the one hand, a number of careful observers working with a large material have failed to find any definite relation between malaria and a positive Wassermann reaction; on the other, a still larger number of clinicians have seen, in a considerable percentage of malarial cases, a Wassermann reaction, strongly positive during the attack, becoming permanently negative after an interval of from six days to six weeks after defervescence. These cases can hardly all have been syphilitic, nor is it easy to explain the findings as due to technical error. The divergence of the results still awaits explanation.

Several of the clinicians[9, 10, 17] who have obtained positive Wassermann reactions in cases of malaria, report a similar experience with the flocculation tests, especially those of Sachs-Georgi and Meinicke. The Kahn test also has been found to give false positives in malaria. Curth,[3] who himself worked only with the Wassermann test, states that he is informed that the Kahn test is no longer used at the Hygienic Laboratory and at the General Hospital in Guatemala City because of frequent falsely positive reactions in cases of malaria which is very prevalent there. Sabatucci[18] considers that of all flocculation reactions for syphilis the Kahn test gives the greatest number of false positives in malaria. On the other hand, Smith,[19] working in the Amazon River Valley, reports results with the Kahn test almost identical with those which Saunders and Turner obtained with the Wassermann reaction. He did Kahn tests on the blood of 194 patients acutely sick with malaria. Of these patients, 40 per cent showed a positive Kahn. However, owing to the prevalence of clinical syphilis and yaws, the incidence of positive Kahn tests in the general population is almost exactly the same, since in 1,099 unselected cases, a positive Kahn reaction was found in 452, or 41 per cent. Of the positive Kahn reactions in malarial patients, 45 were repeated after three to six weeks, when the patients were clinically well. The Kahn tests became negative in none, and in only 2 was there a slight decrease in intensity. He concludes: "The specificity of the Kahn test is not affected by acute malaria or its febrile reaction."

Our own interest in this question was aroused by the following rather embarrassing case. Mrs. T. came to the office complaining of vague malaise, chiefly vertigo with headache, weakness, and a chilly feeling. The temperature was normal; physical examination was negative; there was no splenomegaly. The urine contained 3.5 per cent sugar, and a routine blood Kahn was strongly positive (four-plus). No blood spreads were made. A second tube of blood was sent to a clinical laboratory, well known for its careful work, which reported the Wassermann and Kline tests each four-plus. The patient and her husband were summoned to the office and the nature of syphilis explained to them and the importance of a prolonged course of treatment pointed out. Ten days later she returned stating that she had had a chill two days before and again just before coming to the office. Her tem-

perature was 105° F.; there was no splenomegaly, but the blood contained the parasites of tertian malaria. Quinine therapy was instituted. Two weeks after defervescence, the Kahn test was still slightly positive (one-plus), but two weeks later it had become negative. Since then, at two- and six-month intervals, the Kahn test has remained negative, and the patient has been well except for a mild diabetes.

Malaria is not very prevalent in St. Louis, but during the past four years, 61 nonsyphilitic cases of malaria came under observation in the private and hospital practice of one of us. The other during 1935 studied 93 cases in the wards of the City Hospital in which Kahn tests could be done. The great majority were of the tertian type, a few being estivo-autumnal. All cases in which there existed even a suspicion of syphilis were carefully excluded. Of these 154 cases, 37 gave a positive Kahn reaction (usually a four-plus), making over 21 per cent positives. In 28 of the patients with a positive Kahn, a Wassermann test was also done: 22 of the sera gave a positive reaction; 6, a negative. No case was seen in which the Wassermann test was positive while the Kahn test was negative.

Of the 37 malarial patients with a positive Kahn test, 13 could be followed up serologically. In all of them, the Kahn test became negative after quinine therapy had caused cessation of fever. In 4 cases, the Kahn test had become negative within eight days, in all of them within fifteen days.

One case is interesting from another angle. F. C. came to the office with a history very suggestive of malaria. She had taken some quinine but had discontinued it on account of vomiting. She was running a little daily fever and had a large spleen. The blood showed a marked secondary anemia, but repeated thick and thin spreads failed to reveal the presence of malarial parasites. The blood Kahn test, however, was three-plus. Under quinine medication, the fever ceased; the spleen became smaller and finally could no longer be felt; the Kahn test two days after defervescence was one-plus, fifteen days later, negative, and has remained so. It would seem that occasionally a positive Kahn test might aid in the diagnosis of malaria.

The most striking testimony against the view that malarial infection will cause a positive Kahn or Wassermann in a considerable proportion of cases has been that of Smith and of Saunders and Turner, who found the percentage of positive serology in malaria identical with that in the general population. In our group of patients, this was not the case. In the private practice of one of us, it has been routine to do a Kahn test on the blood serum of each new patient. During the past two years, out of 679 such patients, 37 showed a positive Kahn test, making 5.4 per cent. In the medical wards of the Jewish Hospital, of St. Louis, from which some of our malarial cases were drawn and where the same rule is observed, there were 411 admissions during the past year with 19 positive Kahn tests, a proportion of 4.6 per cent. In the City Hospital, of St. Louis, the proportion of positive Kahns is higher, being about 15 per cent of the total population. However, among the cases of malaria there, without regard to the presence of syphilis, the percentage of positive Kahn tests was 31 per cent. The difference, or 16 per cent, may reasonably be regarded as indicating the incidence of positive Kahn tests due to malaria.

SUMMARY

1. A review of the literature reveals a wide discrepancy regarding the occurrence of positive Wassermann and Kahn reactions in malaria. In the former test, one might possibly explain this discrepancy on the basis of variations in technic. The Kahn test is, however, so thoroughly standardized that such an explanation is not valid here. It seemed, therefore, worth while to investigate the frequency of positive Kahn tests in malarial infections.

2. In 154 cases of malaria free from the suspicion of syphilis, drawn from the practice of one of us and from the wards of the Jewish Hospital, of St. Louis, and the St. Louis City Hospital, the percentage of positive Kahn tests was 21 per cent.

3. The percentage of positive Kahn tests in the general population was 5.5 per cent in the private practice of one of us and 4.6 per cent in the medical wards of the Jewish Hospital. In the City Hospital, the percentage was 15 per cent.

4. It seems clear to us that in St. Louis, at any rate, malarial infection offers a possible source of error in the interpretation of the Kahn test.

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OIL ASPIRATION PNEUMONIA*†

Report of Two Autopsied Cases in Adults

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Introduction.—Attention has been directed to oil aspiration pneumonia on this continent largely through the contributions of Laughlen,[1] Pinkerton,[2, 3] Rabinovitch and Lederer,[4] Ikeda,[5, 6] and Graef.[7] These workers have reported a total of 31 autopsied cases among which 26 occurred in infants or children and 5 in adults. Perusal of the literature will reveal a number of additional reports, most of them concerning clinical cases.

Etiologic Factors.—The various circumstances or mechanisms predisposing to the development of this disease have been discussed thoroughly by the authors mentioned above. A point in common among many of the reported cases is the presence of some interference with the act of swallowing, due either to local or central causes. Infantile marasmus, stuporous states, and central nervous system lesions are often contributory. Any depression of bulbar function sets the stage for aspiration of oil into the lungs. The methods by which the oil is introduced differ, but it is known that intranasally instilled oily medicaments, and orally administered cod liver oil or mineral oil account for a high percentage of the cases reported. The forced intake of cod liver oil into the throats of resistive children has been responsible for the development of oil aspiration pneumonia. Fatty substances present in foods have not infrequently been etiologic in infants.[8] Although certain writers have stressed the point that oil aspiration pneumonia is not a disease liable to affect healthy people, it should not be thought that this is a hard and fast rule. The existence of impaired deglutitive function is not absolutely necessary in order that oil be aspirated, particularly when the oil is taken intranasally. Graef[7] has called attention to the fact that mineral oil is non-irritating and does not excite the cough reflex. The addition to it of mildly anesthetic substances such as menthol further lessen its irritative potentialities. Along this line, the experience of Tchertkoff and Ornstein,[9] as set forth in a recent clinical report, is interesting. These authors reported 10 cases of "bronchopulmonary disease" which they attributed to the intranasal use of oily substances. Eight of their cases were encountered during the space of a few months of consultant office practice. The authors warned against the indiscriminate use of intranasal oil and stressed the importance of questioning chronic lung sufferers as to whether or not they used nasal medication, in order to detect the disease more frequently. All of their patients improved

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†This term, suggested by Ikeda, is used here to include any lung disease attributable to the aspiration of oils or fats.

when the intranasal medication was stopped. What might result if intranasal instillations of oil are persistently and frequently used over a long period of time is illustrated in a case reported by Fischer-Wasels.[10] His patient died of heart failure secondary to extensive chronic pulmonary fibrosis. She was known to have taken oil intranasally for twenty years. One hundred cubic centimeters of mineral oil were recovered from her lungs postmortem.

REPORT OF OUR CASES*

Two additional autopsied cases of oil aspiration pneumonia encountered in this institution during 1935 are herewith reported, not so much because of the supposed rarity of the condition, but rather in view of the interesting factors predisposing toward the aspiration of oil in each case.

CASE 1.—*History.*—W. C., an unmarried white man, fifty-eight years of age, a glass blower by occupation, was admitted to the General Hospital June 19, 1935, because of inani-

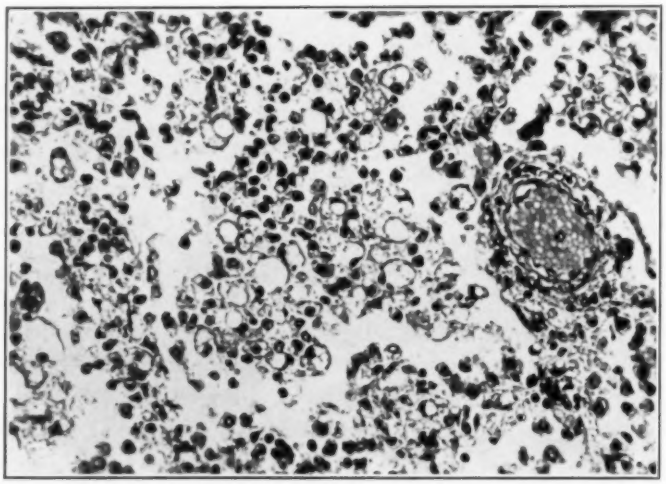

Fig. 1.—Section showing oil-containing macrophages within air sacs (Case 1). Hematoxylin and eosin stain: ×250.

tion and general weakness. He gave a history of occasional "blind staggers" and "dizzy spells" during the preceding few years. Examination showed him to be emaciated, apathetic, and mentally confused. Neurologic examination was negative.

During his stay in the hospital he was up and about until two weeks before his death. He often had difficulty in swallowing either solid or liquid foods. He received haliver oil in 15 minim doses 3 times daily. On two occasions (June 15, 1935 and July 20, 1935), he was given mineral oil in 0.5 ounce doses. On August 5 he began to cough and steadily became weaker. He died Aug. 13, 1935.

Autopsy.—Gross Findings: The body was emaciated. In the lower lobe of the right lung were several rounded areas of consolidation, each about the size of a walnut. Each presented a grayish red, slightly convex, granular, greasy cut surface. The gallbladder contained a few stones. Both kidneys were moderately contracted, with coarsely granular cortical surfaces. (The brain was removed and examined but was entirely negative.)

Microscopic Description of Right Lung.—A fairly acute reaction to the aspirated oil was present in the consolidated portions of the right lung. In frozen sections, the oil was seen to be distributed in tiny droplets within swollen macrophages. The oil stained yellow

*Irrelevant or negative clinical and laboratory findings have been omitted.

with scarlet red and failed to react with either nile blue sulphate or osmic acid. In paraffin sections, the oil-containing macrophages or "lipophages" were easily identified by their vacuolization (Fig. 1). Many of the cells were greatly swollen. In some almost no cytoplasm remained. Nuclei were often eccentric. These oil-containing cells were grouped within the air sacs, often in company with one or more other exudative elements, chiefly fibrin, serum, and polymorphonuclear leucocytes. Many air sacs were completely filled, solely with lipophages. In others there was an admixture with fibrin or polymorphonuclear leucocytes or both. In yet other areas the response was serous or serocellular. In the peripheral portions of the consolidated areas, the exudate was predominantly fibrinopurulent. An interesting finding, several times noted previously in this disease, was that in many of the alveoli which contained lipophages, the alveolar epithelium was easily demonstrable, being made up of deep-staining cells of cuboidal to low columnar type.

Although for the most part the reaction to the oil was acute, in several areas concentric formations of young fibrous tissue were seen enclosing large droplets of oil which were intimately clothed by foreign body giant cells. Other large droplets of oil, which seemed to have resulted from the agglomeration of clumps of lipophages, were surrounded by deep staining

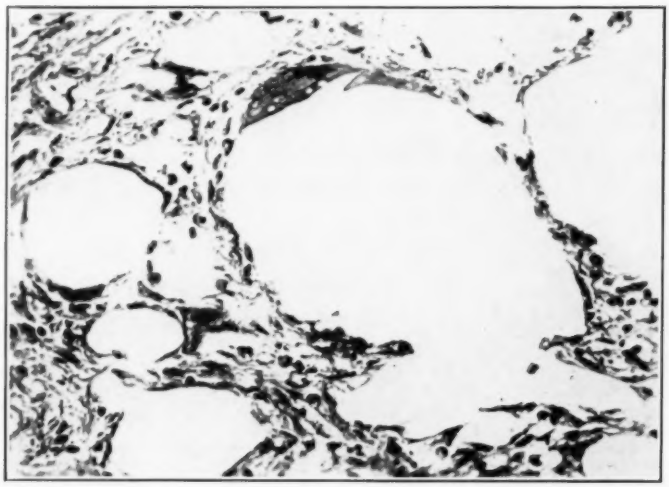

Fig. 2.—Section showing foreign body granulomatous reaction to large oil droplets in scarred lung (Case 2). Hematoxylin and eosin stain; ×250.

fresh macrophages which seemed to be in a process of fusion to form foreign body giant cells. These droplets had not yet excited fibrous response.

Diagnosis: Acute bronchopneumonia (mineral oil aspiration); inanition; chronic glomerulo and interstitial nephritis.

CASE 2.—*History:* E. L., a married white man, sixty-one years of age, a bug exterminator by occupation, was admitted to the General Hospital Oct. 15, 1934. In addition to chronic arthritis of both knees, he complained of shortness of breath. He remained in the hospital for nearly a year, during which time he was usually able to be up and about. He showed mental confusion, continually drooled at the mouth, and had difficulty in swallowing. He was frequently constipated, but when laxative tablets were given him, he would hold them in his mouth until he felt he was not watched, when he would spit them out. On June 11, 1935 he suffered a mild cerebral accident characterized by muscular twitchings of the face and both upper and lower extremities. There were no residual signs. From this time on, he was given mineral oil in one ounce doses when indicated, rather than pills or tablets. Up to the time of his death he had received at least 10 such doses; this medication too he held in his mouth and later spit out if not carefully watched.

On Sept. 6, 1935 he sustained a severe cerebral accident, causing left-sided hemiplegia. He became comatose and died Sept. 7, 1935.

Autopsy.—Gross Findings: There was an extensive right-sided cerebral hemorrhage; severe atherosclerosis was present in the cerebral and coronary arteries and aorta. A patchy bronchopneumonia was present in the lower lobes of both lungs and in the right middle lobe. The larger branches of each pulmonary artery contained loose embolic chunks of thrombus material. The heart was dilated; mural thrombi were present in the right auricle. The kidneys were slightly contracted.

Microscopic Description of Lungs.—Several of the consolidated foci presented an acute seropurulent or serofibrinopurulent exudate. A block from another place presented an early reaction to oil, quite similar to that described in Case 1. In addition, there were several foci of fibrotic reaction, much older than any seen in the first case. In these areas, the lung architecture was replaced by dense fibrous tissue which was honeycombed by large and small oil droplets (Fig. 2). Each oil droplet was bordered by foreign body giant cells. In frozen sections, all of the oil stained yellow with scarlet red and failed to react with osmic acid and nile blue sulphate.

In this case, the appearance of definite columnar epithelium lining the alveoli, which were filled with lipophages, was more pronounced than in the preceding one.

Diagnosis: Cerebral hemorrhage; acute and chronic bronchopneumonia (mineral oil aspiration); pulmonary embolism; generalized arteriosclerosis.

COMMENT

The intrapulmonary lesions observed in both of these patients were quite similar morphologically to those previously described by other writers, notably Laughlen,[1] Pinkerton,[2, 3] and Graef.[7] All of the stages of pulmonary reactivity to oil were encountered in one or the other of these two cases, ranging from intraalveolar phagocytosis of fine oil droplets early in the process, to the formation of densely fibrous foreign body granulomas about large oil droplets late in the disease. In both cases the oil found in the lungs gave staining reactions typical of mineral oil. This would indicate that in Case 1 the haliver oil did not gain entry into the patient's trachea, even though he had had many doses of it, and only two of mineral oil. An explanation may lie in the fact that haliver oil is more irritant to the pharynx and trachea than mineral oil. In each case there was an associated septic bronchopneumonia, an expected finding in the light of previous reports.

From a practical standpoint, a valuable lesson is contained in these two cases. Neither patient suffered from demonstrable paralyses during the time the oil was administered. The only clinical contraindication to the oral administration of mineral oil presented by these two patients was their difficulty in swallowing. This impairment of the swallowing act was not marked, as neither had to be hand fed. One patient's (E. L.) practice of holding the oil in his mouth for a time in order to avoid swallowing it, certainly may have further disposed toward the aspiration of oil in his case. It is probable that the incidence of oil aspiration pneumonia could be materially lessened if physicians would routinely question patients regarding dysphagia before prescribing mineral oil for laxative purposes.

The attention of practicing physicians is not as yet sufficiently focused upon the problem of oil aspiration pneumonia, not only as a morbid entity, but, just as important, as an insidious and dangerous clinical ailment, which like many other diseases, if unsuspected remains undiagnosed. Oil aspiration

pneumonia is not necessarily fatal. All of the patients reported by Tchertkoff and Ornstein[9] improved when intranasal oil medication was stopped. In all probability, individuals may recover from mild or subclinical lung damage resultant from administration of oil by mouth, without the condition's ever having been recognized. The suggestion of Rabinovitch and Lederer[4] that sputum from suspected cases be examined for oil-containing monocytes seems a good one. This procedure along with careful history-taking and adequate x-ray studies should lead to more frequent clinical recognition of this interesting and often unnecessary disease.

CONCLUSIONS

1. Two cases of oil aspiration pneumonia in adults are herewith reported with autopsy findings. The pulmonary lesions observed in each instance correspond well with those described by the writers mentioned previously.

2. Oil aspiration pneumonia is often preventable. Care should be taken that mineral oil as a laxative should be prescribed only for those patients in whom there is no interference with the act of swallowing. The frequent use of nose oils, to judge from certain recent contributions to the literature, is also fraught with danger, even where there is no impairment of the deglutitive function.

3. If increased attention is directed toward oil aspiration pneumonia, it will be recognized more frequently, both clinically and at autopsy.

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STUDIES ON CONSTITUTION AND PEPTIC ULCER*

IV. Salivary Secretion Test in Peptic Ulcer Patients and Normal Subjects

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EPPINGER and Hess of Vienna applied the conception of "vagotonia" to peptic ulcer patients on the basis that the majority of them presented symptoms attributable to a hyperactivity of the parasympathetic nervous system, but admitted that in a number of ulcer patients mixed symptoms were to be seen. They injected peptic ulcer patients with pilocarpine, and reported an increased salivary secretion in response to this drug.[1] Von Bergmann and his school elaborated upon the idea of Eppinger and Hess, and supplanted the conception of vagotonia by that of "autonomic imbalance," by which they characterized ulcer patients.[2, 3] Increased salivation in patients with peptic ulcer has been described by several authors.[3-5] None of these investigators, however, have made a comparative study on the salivary secretion of ulcer patients with that of a similar group of normal persons.

MATERIAL AND METHODS

All subjects were white adult males between the ages of twenty and sixty-six years. Thirty normal individuals free of any gastrointestinal complaints were selected, along with 34 peptic ulcer patients picked at random from our gastrointestinal clinic. Two of the patients suffered from gastric ulcer, the remainder from duodenal ulcer. The ulcers were in various stages of activity. The diagnosis had been established clinically, roentgenologically, and by other laboratory tests, in several by operation; no case was included in which the diagnosis was doubtful.

The individuals presented themselves for the test in the morning, two or three hours after an ordinary breakfast. A subcutaneous injection of 1 mg. of pilocarpine nitrate for every 30 pounds of body weight was given. The expectorated saliva was collected for an hour following the injection, and the volume measured. In all cases the peak of the secretion curve occurred in the second quarter-hour period. No untoward reactions from the pilocarpine injection were observed, but nearly all subjects experienced some flushing and perspiration.

The data were tabulated in the form of a correlation table, the coefficient of correlation between the volume of secretion and age being determined according to the formulas of Pearl.[7] Statistical means and the significance of their differences were computed by the method of Fisher.[8]

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RESULTS

From Table I it appeared that the older group of ulcer patients (forty-one to sixty-six years) secreted more saliva than the younger group (twenty-one to forty years), and that no such difference existed between the normal groups. By using Fisher's formula[8] for small series, it was found that the increase of salivary secretion with age in the ulcer group was not significant. Therefore, the combined age groups of the ulcer series were compared with the combined

TABLE I

AGE IN YEARS	NO. OF SUBJECTS		SALIVARY SECRETION IN C.C.	
	ULCER	NORMAL	ULCER	NORMAL
21-40	13	13	86	115
41-66	21	17	102	118
21-66	34	30	94.1 ± 6.4	116 ± 7.3
Difference of the means of both groups and standard error of the difference			21.9 ± 9.7	

age groups of the normal series. The means of all ulcer patients and of all normal persons determined statistically were 94.1 and 116 c.c., respectively. The difference of the means of the normal and ulcer group was 21.9 ± 9.7 c.c. Since this difference was more than twice its standard error, it had to be considered significant, and it must be concluded that peptic ulcer patients secrete less saliva following pilocarpine than normal subjects.

DISCUSSION

The finding that the patient with peptic ulcer secretes less saliva to pilocarpine stimulation certainly speaks against the theory of generalized vagotonia. Our results do not necessarily disprove that ulcer patients may have increased salivation (Winkelstein[4]), but the probability is, that it is not so. In a previous paper of this series, we reported that gastric appetite secretion was higher in acidity in ulcer patients than in normal subjects.[9] It was pointed out that the greater acid response to appetite stimulation in ulcer patients could hardly be used diagnostically, because the individual results were too scattered and only statistical treatment proved that there was a significant difference. The same is true in the present paper. The significance of the difference in salivary secretion between the normal and ulcer group had to be proved statistically, and therefore salivary response to pilocarpine cannot be recommended as a simple diagnostic test.

It remains an open question why the ulcer patient secretes less saliva than the individual without an ulcer. Many patients with gastric ulcer display hypermotility of the stomach and many patients with peptic ulcer of the duodenum show gastric hypermotility and hyperacidity. It is probable that this hyperactivity is due to impulses reaching the stomach through the vagi or by a humoral mechanism. One may assume, then, that certain centers in the midbrain are in a state of hyperactivity (centers for gastric secretion and motility), and that other centers are not affected, or even may be depressed (salivation center). Since salivary secretion is a complex process, one must think also of psychic inhibition or of peripheral reflex inhibition.

The work presented tends to show that peptic ulcer is not a local disease, but probably a systemic one. In another paper of this series, it was shown that the supposedly healthy members of ulcer families have an abnormal gastric secretion,[10] so that constitutional differences do exist between ulcer patients and their relatives and normal individuals.

Our results are not in favor of the theory of vagotonia, but support the concept of autonomic imbalance, somewhat because a disturbance (or imbalance) in the parasympathetic system itself may explain our findings.

SUMMARY

The salivary response to pilocarpine was determined in a group of 30 normal persons and 34 ulcer patients. In neither group was any relation of volume of secretion and age seen. The salivary secretion of the ulcer group was significantly lower than that of the normal control group.

We are indebted to Dr. T. O. Yntema, School of Business, University of Chicago, for advice in the statistical treatment of the results.

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LABORATORY METHODS

THE GONOCOCCUS COMPLEMENT FIXATION TEST*

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SEVEN years before the discovery of the gonococcus by Neisser, Noeggerath[1] attracted the attention of physicians to the importance of latent gonorrhea in the female in his masterly clinical study entitled *Die latente Gonorrhoe des weiblichen Geschlechts.* Since the etiologic agent of gonorrhea was as yet unknown, Noeggerath's statements were based only on clinical observations. The difficulty in diagnosis encountered by this worker is obvious.

With the discovery of the gonococcus, the difficulty in diagnosis of subacute and chronic cases of gonorrhea was further emphasized. Frequent microscopic examinations sometimes had to be made before gonococci could be found in smears. Zill, as cited by Brandstrup,[2] states that gonococci may be demonstrated in females in 40 per cent of chronic cases after an examination of seven smears; in some instances it has been possible to demonstrate the gram-negative diplococci only after examining 70 to 80 smears.

With the adoption of the culture method as routine, there seemed to be some increase in the efficiency of diagnosis of gonorrhea. It is evident that material containing only a few organisms will yield a greater number of positive results when examined by the cultural than by the microscopic method, because of the increase in numbers due to growth in a suitable medium. An experienced worker in this field knows that there are instances in which only the smear may be positive while the repeated cultures fail to show growth of the gonococcus. Therefore it is essential to employ both methods as aids in the diagnosis of gonorrhea.

Even these laboratory procedures are limited in their usefulness, particularly in the cases in which the gonococci have penetrated into the tissues and surrounding structures so as to disappear completely from the discharge.

The lacunae of Morgagni and the glands of Littré of the male anterior urethra, Cowper's glands, the prostate, the seminal vesicles, or the epididymis frequently become encapsulated foci of gonococci. Depending on the stage of inflammation, these foci may be more or less surrounded by an inflammatory cell and tissue reaction or may even form an abscess. This type of latent gonococcal infection is also known as a frequent complication of the disease of the female sex organs. R. Schroeder,[3] among others, has demonstrated gonococci in the deeper layers of the altered mucous membranes of the cervix, concealed in the interstitial infiltrations of round and plasma cells lying between the deep-

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est positions of the cervical glands. Only rarely were the deeper uterine tissues found to be involved, since its periodic change and power of regeneration, as the result of ovarian hormone stimulation, protect this organ, in some degree, from an extensive infection. The tubes and ovaries act as reservoirs or foci par excellence for the gonococcus. Latency of the infection is therefore a frequent sequel in both the male and female genital organs.

As smears and cultural procedures are not always of value in the diagnosis of latent gonorrhea, the complement fixation test that was discovered in 1906 by Müller and Oppenheim,[4] and, independently of them, by Bruck,[5] becomes useful as an aid in diagnosis of gonococcal infection. Many investigators in this country, among them Teague and Torrey,[6] Wollstein,[7] Schwartz and McNeil,[8] and a great number in Europe, including Vannod,[9] Krumbein and Schatiloff,[10] and many others were among the pioneers of this new laboratory method. Because of the technical difficulties and errors in interpretation, the use of the complement fixation test as a routine laboratory procedure was delayed for more than twenty years. The main difficulty in the past has been the production of a suitable antigen that had a wide range of reactivity. Attempts to improve the gonococcus complement fixation technic have been only moderately successful, since it is far more delicate than the Wassermann test. The reaction has been improved in many ways but much more still remains to be done. It was not until seven years ago that the European countries accepted it as a routine method of diagnosis. This aroused in this country a new interest in the reaction. Considering the American literature of the last five years, the reader is confronted with different opinions in regard to the clinical value of the reaction. There are some workers who think that this test has a very limited, if any, clinical value (Herrold,[11] Jacoby,[12] Bucher,[13]) while others (Kolmer,[14] Swan,[15] Barringer and her associates,[16] Brunet and Levine[17, 18]) have reached the opposite conclusion. Barringer, Strauss and Crowley mention the importance of this method for the determination of a cure. This matter has been under discussion for many years among the European investigators. In a paper on this subject, published by me together with Gräfenberg[19] in 1925, it was pointed out that this test was probably helpful in making more certain the establishment of cure. In spite of extensive work this problem still remains unsettled.

The purposes of the study reported here are: (1) to help the clinician in the interpretation of the gonococcus complement fixation test with respect to its clinical value and to demonstrate the limitations of the reaction; (2) to prove that this test is helpful in the determination of cure.

EXPERIMENTAL

The technic which has been used in these studies is the same as that which has already been published. The main principles of this procedure will be repeated here.

Antigen.—The gonococci (about 12 strains) were originally cultivated on Levinthal's cooked blood agar, but are now grown on the modified filtrated cooked blood agar, suggested by Bayne-Jones. The age of the strains is of minor importance, since old laboratory strains show the same antigenic power as young

ones. It is very important to have strains with a wide antigenic reactivity. From the experiments of Teague and Torrey and the more recent study of Tulloch,[20] it is known that a main or "predominant" type of the gonococcus causes infection in 72 per cent of all cases. The remainder of the organisms are divided into four smaller groups, which are less frequently the causes of infections (28 per cent). It has been reported in a previous paper[21] that a predominant strain, which Tulloch received from Teague and which was sent to me, had nearly the same antigenic reactivity in Berlin as in this country and in England. So, it probably is sufficient for practical purposes to use an antigen made with several strains in order to insure the presence of the predominant type in the antigen and to avoid typing of the gonococci.

Forty-eight- or seventy-two-hour cultures of gonococci, which have previously been identified, are examined for purity. Five cubic centimeters of normal saline are added to each Petri dish culture and the colonies carefully suspended by streaking the loop over the surface of the medium. To avoid nonspecific action of the antigen, care is taken not to include any of the agar medium. The suspensions, to which 0.5 per cent phenol is added, are collected in liter bottles and closed by glass or rubber stoppers, since cork or cotton plugs may influence the specificity of the reaction. It is advisable to store the bottles in the ice box for about three months and to shake the suspensions occasionally in order to "ripen" the antigen properly.

After three months the antigen is ready for titration. To secure a constant titer the suspension must be shaken each time before use. The gonococcus antigen is mixed in amounts ranging from 0.1 to 0.025 c.c. with known positive and negative human serums, diluted 1:5, as well as with normal saline as a control. After adding complement in a dilution of 1:10 to each tube, the whole test is incubated at 37.5° C. in the incubator for one hour. The use of the incubator is preferred because the reaction in the water-bath proceeds too rapidly. After addition of the hemolytic system, the test is reincubated and read as soon as the control tubes (serum without antigen, and negative serum with antigen) are hemolyzed. The titer of the antigen is considered as the lowest dilution which gives a reaction with the positive serum and complete hemolysis with the negative serum. In most cases the antigen-saline test tube will also be hemolyzed in the corresponding dilution; because of the fact that the anticomplementary quality of the antigen is neutralized by the presence of serum, this control is not regarded as essential. The antigen must be tested with many serums before the exact titer is determined. As a rule the titer lies between 0.06 and 0.03 c.c. for a total volume of 0.25 c.c. It must be emphasized that only human serums should be used for titration, since immune serums of animals contain antibodies in higher titer and therefore require less antigen for the test. The titer determined by the above method usually remains constant. An increase of the antigenic power within the first six months, followed by a slight decrease, may occur.

Serum to Be Tested.—The patient's serum is obtained in the same manner as for the Wassermann test. If the blood is to be shipped, it is also essential to close the glass tube with a rubber stopper. Soon after the arrival at the laboratory the blood must be centrifuged, the serum inactivated at 56° C. for

half an hour, and then stored in the ice box for twenty-four hours. Since freshly inactivated serum shows an increased lability of its reactive substances and therefore might yield false serologic results, the test should not be made on the day of inactivation. A serum used more than three days after being taken from the patient may give nonspecific reactions. The same is true for serums from patients who have recently been immunized with gonococcus vaccine; a period of at least six weeks must elapse before results, not influenced by the immunization, can be expected.

Amboceptor.—The titration of the amboceptor is carried out in dilutions of 1:200, 1:400, et., to 1:25,600. Complement taken twenty-four hours previously from a healthy male guinea pig in a dilution of 1:10 and sheep's blood in a dilution of 1:20 are added. The different reagents are always used in the same amounts (0.25 c.c.). Each tube contains a total of 1.25 c.c.; the difference, due to lack of serum and antigen in this preliminary test, is made up with normal saline. Two control tubes containing no amboceptor are set up in order to exclude the possible hemolytic action of the complement or the saline. This preliminary test is incubated at 37.5° C. for one hour. The first reading is made after twenty minutes, the final one after one hour. The unit of amboceptor is taken either as that dilution in which complete hemolysis occurs in twenty minutes, or one-fourth of that dilution in which complete hemolysis occurs in one hour; the lowest dilution showing hemolysis is taken as the titer. For example: if after twenty minutes complete hemolysis occurs with 1:800 dilution of amboceptor, after one hour with a 1:3,200 dilution, the unit is 1:800. Or, if hemolysis occurs after twenty minutes in a dilution of 1:400, after one hour with a dilution of 1:3,200, the unit is 1:400. Or, if hemolysis takes place after twenty minutes at a dilution of 1:1,600 and after one hour at a dilution of 1:3,200, the unit is 1:800. The purpose of the double reading at different intervals of time is to exclude the hemolyzing power of the complement itself.

Main Test.—In the main test three tubes are used for each serum. The first, which acts as a serum control contains no antigen but double the amount of serum (0.5 c.c.). The second and third tubes contain all the constituents of the first tube with the addition of the antigens which differ in each tube. Known positive and negative serums are always included in the main test as controls. After adding complement, the test is incubated at 37.5° C. for one hour. The hemolytic system (0.5 c.c.) is then added to each tube. Readings are made as soon as the negative serum controls, with and without antigen, are hemolyzed. This may take place in from twenty to sixty minutes. With readings at the moment when the controls are hemolyzed, the weak positive reactions are not overlooked.

The complement fixing power between antigen and antibody is the weak point in this test, a fact which can readily be shown by comparison of the gonococcus complement fixation reaction with the more stable Wassermann test. The Hamann combined system, reported by Thomson, Hamann and Park,[22] attempts to make the test more stable by titrating the fixing power of the complement.

The tests reported in this paper were carried out, using the technic which has been described above and the results recorded as ++++ (strongly positive);

++++ (positive); +++ or ++ (weakly positive or doubtful; the weakly positive or doubtful reactions can only be satisfactorily interpreted on the basis of history, clinical and bacteriological findings); – signifies the absence of any complement fixing antibodies.

While ++++ and +++ reactions are considered to be indicative of the presence of a gonococcal infection, the weak positive reactions (++ and +) are not evidence of disease if a positive history, smear or culture cannot be obtained.

RESULTS

Comparison of Serologic Results and Clinical Diagnosis.—The material used in the study presented here was obtained, in its greatest part, from patients in the New Haven Hospital. A smaller number of serums were furnished through the kindness of Dr. M. J. Strauss from the New Haven Municipal Clinic and other institutions; 1,153 tests of 495 patients (470 adults, of whom 199 were males and 271 females, and 25 children, of whom 24 were females and 1 male) have been performed. Since material for routine diagnosis was taken from individuals who had suspicious clinical signs, but in whom no history or bacteriologic evidence of gonorrhea could be found, there is a large number of negative reactions in this group. On the other hand, patients who had definite bacteriologic gonorrhea were tested in various stages of the disease. Many single serums were examined numerous times, in order to study the stability of the reaction in a single specimen. In several instances serum was taken from the same patient at different times in order to determine both the constancy of the reaction and the increase or decrease of complement fixing antibodies. It has been impossible to test some of the serums, because they were either hemolyzed or showed a strong anticomplementary action.

Table I, in which the clinical diagnosis and the serologic findings in each case are presented, demonstrates the relation between the clinical progress of the disease and the increase of complement binding antibodies. The cases are grouped according to sex, where the disease was not systemic in nature. The presence of gonococcal infection in all of the patients was determined bacteriologically. The figures and percentages presented here are not so high as some recorded in the literature, because cases of early and late infections have been included; no attempt was made to determine the maximum reaction in any single case.

Some explanation of the reactions which show a complete absence of complement fixing antibodies seems necessary. It is possible that the antigen used in the test does not contain the strain which has caused infection in any particular negative case. Since 72 per cent of all cases of gonorrhea are the result of infection with a "predominant type," this explanation will hold only rarely. Another possible explanation is that a patient may have lost the power of producing antibodies of any sort (last stage of infection, malignant tumors, etc.). A more frequent reason, however, is that the duration of the infection is too short for antibodies to be produced. As in the Wassermann reaction, the gonococcus complement fixing antibodies are not found before the end of the second or third week. Many investigators believe that they can elicit the reaction

earlier than this by improving the technic of the test. Price[23] tried to demonstrate this fact by immunizing himself, patients and animals with a gonococcus vaccine. He found a strongly positive serologic reaction present by the tenth day. However, the mechanism of antibody production seems to be different from that of natural infection. The problem involves the question as to whether or not it is possible to use this test in making a diagnosis of early gonorrhea.

TABLE I

RESULTS OF COMPLEMENT FIXATION TESTS IN VARIOUS CASES

REACTION	++++		+++		++		+		0	
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
A. Males:										
Anterior urethritis 50 cases	2	4	2	4	7	14	1	2	38	76
Posterior urethritis 83 cases	10	12.05	16	19.27	22	26.50	20	24.10	15	18.07
Prostatitis 7 cases	2	28.57	2	28.57	2*	28.57	-	-	1	14.29
Epididymitis 7 cases	4	57.14	1	14.29	2*	28.57	-	-	-	-
B. Females:										
Cervicitis 30 cases	7	23.33	5	16.66	3	10.00	2	6.66	13	43.33
Adnexitis 18 cases	11	61.11	2	11.11	3	16.66	1	5.55	1†	5.55
Bartholinitis 4 cases	1	25.00	-	-	2	50.00	1	25.00	-	-
C. Arthritis: 7 cases	4	57.14	1	14.28	-	-	-	-	2‡	28.57
D. Chronic: 9 cases	2	22.22	2	22.22	3	33.33	2	22.22	-	-

*Changed later to ++++.
†Changed after twelve days to ++++.
‡One: ten days later to ++++; second, after eighteen days to ++.

Cohn and Gräfenberg pointed out this possibility in 1925, but after further studies the idea was dropped, because of the fact that an early formation of antibodies in gonococcal infections could be demonstrated only irregularly and exceptionally. In the present study serum from a patient suffering from an acute gonorrheal discharge for five days was found to give a positive reaction; the clinical diagnosis was acute infection or recurrence. Nevertheless, because of a strong suspicion that these symptoms represented a recurrence, this example cannot be cited in favor of early serologic diagnosis. Early diagnosis by the serologic method has no practical importance, since infection can easily be determined by bacteriologic means.

Another frequent reason for the absence of complement fixing antibodies is the nonabsorption of antigen due to the limitation of the infection to the surface of the urethral or cervical mucous membranes. For this reason, it is not surprising that the largest number of negative reactions are found in cases with anterior urethral and with cervical infection. Should antigen be absorbed because the gonococcus spreads or penetrates into the deeper layers of the mucous membrane, which have a greater blood and lymph supply, antibodies will be produced. On the contrary, when treatment is successful and the in-

fection remains localized or is cured, no antibody production occurs. In other words, the clinical conclusion that anterior gonorrhea in the male is more easily cured than cervicitis in the female is corroborated by the different percentage of positive reactions obtained with serums taken from patients suffering with these respective types of infection.

The parallelism which exists between serologic and clinical evidence is also seen in cases of posterior urethral gonorrhea. The number of strongly positive reactions is three times as large as in anterior urethral gonorrhea. This increase in antibodies is therefore due to the spread of the infection, which, as is known from clinical experience, is frequently followed by other complications such as inflammation of the prostate, seminal vesicles, epididymis, or joints. There have been a number of cases of posterior urethritis, which showed a negative or only weakly positive reaction. It is possible that the antibodies disappear with treatment or that the infection runs a mild course, and consequently no antibodies are produced.

The greater the spread of the infection, the larger is the percentage of positive and strongly positive reactions. Cases of epididymitis, salpingitis, oophoritis, and arthritis show the strongest reaction; it is in these stages of infection that the gonococci usually are not found in the smears made from the discharge. The complement fixation test is the only one which can confirm the clinical suspicions in this stage of the disease. Many investigators put much stress on the association of this clinical picture and positive serologic reaction with coincident negative bacteriologic findings; the intensity of the reaction is thought to be the result of the presence of a closed or latent focus. This may be true in many cases, but the same degree of reaction can be found in patients with apparently open foci. Therefore it seems that it is the virulence of the infecting microorganism together with the resulting response of the infected tissue which produces this strong reaction.

The chronic infection confirms this opinion. Similar pathologic changes, such as the formation of closed or latent foci, may be found. In cases of long standing where the response of the tissue to the invading microorganism is weaker, and the blood and lymph supply in the tissue is less, the absorption of antigen is slow and small in amount. The serologic results may therefore be weakly positive (++ or +). Even these reactions must be considered seriously in patients with suspicious histories and clinical findings. Repeated tests and clinical and bacteriologic examinations must be performed. An exacerbation of the chronic process will produce an increase of antibodies, while effective treatment may make the reaction negative. Table I, D shows the small number of chronic cases and weakly positive reactions.

In order to check the results reported by Brandstrup, in which he demonstrated that 33 per cent of pregnant patients with positive reactions had a puerperal fever due to the gonococcus, and in order to determine whether there may be nonspecific reactions in serums from such patients, the bloods of 23 pregnant women were tested. Since most of the cases examined showed a negative reaction, it was impossible to confirm Brandstrup's observations. Twenty of this group had no history or findings of gonococcal infection; 18 serums gave a negative reaction; the other two, a weakly positive (+). Two of the 3

suspicious cases showed a +++; 1 a ++ reaction; in 1 of them the gonococcus was found on culture in the bartholinian gland. The 2 other cases could not be established bacteriologically, but a purulent discharge, temperature in the puerperium, and swelling of the joints in 1 patient, and a discharge, temperature and condylomata acuminata in the other made the clinical picture suspicious.

Four cases of sterility in female patients with negative history and bacteriologic findings gave negative reactions.

The serums of 25 children, of whom 10 showed no evidence of a gonococcal infection, were tested. Nine gave a negative serum reaction and 1 a + reaction; this child was suffering from a septicemia of unknown origin. Of the 15 children who had positive smears and cultures of the vagina or rectum or both, 3 gave strongly positive, 6 a weakly positive, and 6 a negative reaction. These results are similar to those described previously by Cohn and Rosowsky.[24] In these cases no explanation could be found for the discrepancies in the serologic results in children with an identical clinical picture and apparently the same constitutional make-up. The age may be an important factor in this group, since the strongly positive reactions were found in children of three or more years old while the younger ones showed a weak or negative reaction.

The Determination of Cure.—The problem as to whether the gonococcus complement fixation test is useful in the determination of cure has been intensively discussed in European countries for a long time. The surprising fact that positive serologic results were obtained in patients who had been declared cured for many years, and that negative serum reactions were followed by positive bacteriologic findings tended to discredit the test.

Due to the fact that the above-mentioned positive reactions were found in clinically cured cases in which the gonococcal infection had occurred ten to twenty years previously, a persistence of complement fixing antibodies without continued stimulation by an antigen was assumed. The adoption of this idea seems surprising, because the same assumption is never made in the Wassermann reaction, in which similar observations can be noted. Cohn[25] attempted to clarify this point by immunizing normal persons with a gonococcus vaccine. He produced a positive reaction in such persons and found that the antibodies disappeared in from twenty-six days to three months after the vaccination; even if the mechanism of the production of complement fixing antibodies by immunization might be different from that of natural infection, these experiments show, obviously, the disappearance of antibodies after the elimination of the antigenic effect. It is also significant that the persisting positive reactions were found mainly in cases with gonococcal complications which frequently showed a recurrence of the disease. Further, there were cases in which the positive reaction became negative after the clinical cure. All of these facts, taken together, indicate that a continuing positive reaction is more than just the remnant of a serologic reaction.

Unfortunately, the number of serums from patients who were considered as clinically cured, was small. Nevertheless there were cases which show clearly the disappearance of complement fixing antibodies after cure.

Eight cases of anterior gonorrhea, which were thought to be clinically cured, were studied. Seven showed a negative, 1 a + reaction. Since the previous reactions are unknown, no conclusions can be drawn from these cases. Twenty-two patients with a posterior urethritis were examined. The serums of 18 were negative, 1 showed a +++, 2 a ++, and 1 a + reaction. Three serums of this group were tested only once, while the others were investigated several times at different intervals. Of special interest were 6 cases among these eighteen which decreased from a strongly positive (++++) reaction to a negative one within two to four months; the same length of time was necessary for three- and two-plus reactions to become negative. These cases are especially convincing, since the clinical and bacteriologic disappearance of the disease is easier to demonstrate in this stage than in complicated cases. It must be mentioned that in all such instances the clinical diagnosis of cure was made long before the serologic results were obtained.

One cured case of epididymitis and 2 of prostatitis gave a negative reaction, but these had not been tested before.

There were 2 female patients; 1 with a tuboovarian abscess and another with pelvic inflammatory disease. The serums of both of these patients showed a strongly positive reaction, when tested for the first time. Bacteriologic examinations (smear and culture) were performed repeatedly but always gave negative results. The first case became negative after two and a half months. Curiously, a second sample of serum taken after two weeks showed a negative reaction. A third test was again strongly positive, but became and remained negative after two and a half months. The second sample of serum was hemolytic; this may be the cause for the apparently sudden disappearance of the antibodies. A hysterectomy was performed on the second case. The complement fixation test on the day of operation was ++++. Sixteen days later a +++ reaction was obtained, which gradually became weaker until at three months the intensity was +.

The period of time necessary for a positive serum to become negative, if the patient is cured, is from two to four months according to the present observations. This time limit is approximately the same as that found in the above-mentioned immunization experiments. Osmond[26] has reported that, in his experience, positive serums of cured patients fall off very slowly in the strength of the reaction over a period of many months and up to one year.

In order to obtain earlier serologic results for the determination of cure, serums of patients, clinically and bacteriologically cured, have been examined in dilutions of from 1:5 to 1:80. This method showed no particular advantage.

As has been mentioned before, the test does not indicate gonococcal infection in an individual if no response of the infected tissue to the microorganism takes place. This may happen during the course of the disease or late in the infection; in the latter instance, a previously positive serum might become negative and lead to a false assumption of cure. To exclude this source of error, clinical and bacteriologic examinations are necessary. The gonococcus complement fixation test is a valuable aid in the determination of cure when a previously positive

reaction becomes negative and the clinical and bacteriological findings are also negative. Emphasis should be placed on the clinical and bacteriologic findings, since the serologic status alone is not sufficient for a determination of cure.

On the contrary, in cases in which the serum remains positive (+++ or ++++) for a year or longer after the cessation of treatment and the clinical findings are normal or suspicious, every attempt must be made to discover gonococci by help of smears and cultures or other methods.

With respect to the specificity of the test, it has been claimed that from 0.6 per cent to 5 per cent of serums taken from patients suffering with diseases other than gonorrhea may give a positive reaction (++++ or +++) in the gonococcus complement fixation test. Some investigators have demonstrated a positive gonococcus complement fixation reaction with serums from meningitis cases. In the present study two such serums were examined and found negative. Rubinstein and Gauran[27] have claimed that 30 per cent of all Wassermann positive serums give a positive complement fixation test; Martland[28] has observed this in 36 per cent of all Wassermann positive cases. These figures are much higher than those observed in any of our own investigations, because they probably do not take into consideration a concurrent gonococcal infection. The claim that a positive reaction may be found in tuberculosis has not been substantiated in our past or present work; one case of tuberculous salpingitis and one case of tuberculous arthritis which were studied in this investigation were found to give a negative serologic result. Acute infectious diseases with high temperatures, acute skin rashes and generalized dermatitis may give a nonspecific complement fixation test. Repeated serologic examinations together with clinical and bacteriologic studies must be made to exclude nonspecific results.

CONCLUSION

The technic of the complement fixation test, as used in the diagnosis of gonorrhea, has been described. Four hundred and ninety-five cases were studied. The results obtained are given in the tables.

The production of complement fixing antibodies is dependent on the duration and spread of the infection.

In acute complicated cases of gonorrhea the complement fixation test is strongly positive, while in chronic cases, of especially long standing, the reaction is sometimes only weakly positive. Consequently + and ++ reactions must be regarded suspiciously.

The complement fixation test is an aid in the determination of cure if previously positive reactions become negative, and the clinical and bacteriologic findings are also negative.

The negative reaction by itself does not mean the absence of gonococci.

A positive reaction persisting longer than one year after a clinical and bacteriologic cure is a strong indication that a latent focus of the gonococcus is present. Further examinations should be carried out in order to detect the focus.

The complement fixation test is not intended to replace the clinical and bacteriologic methods of diagnosis, but should be a supplementary procedure helpful in the diagnosis and the determination of cure.

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MODIFICATION OF THE GRAM METHOD*

Use of Iodized Decolorizers

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WHEREAS there is a classical method for the Ziehl-staining, which is everywhere employed, there are over a hundred modifications of the Gram method, none of which is generally recognized as being markedly superior to the others. Such a profusion of prescriptions might be explained if on one point of the original method there was a defect, which has escaped attention in successive modifications.

The mechanism of the gram-staining has been analyzed by many workers, and, according to Beniens,[1] the iodine forms with the pararosaniline dyestuffs a complex of high molecular weight, the membrane of the gram-positive bacteria being impermeable to it. Burke[2] has given an experimental confirmation of this hypothesis. But those authors have only considered the diffusion and retention of the iodine complex as if it were thoroughly stable.

But the decolorizer may produce an additional effect. It may dissociate the complex, especially if its action is prolonged. The iodine-methyl-violet-compound appears to be rather loose, and the iodine, which has great affinity for the solvents used as decolorizers, may dissociate and diffuses out progressively, and of course the liberated violet will diffuse likewise.

If this is true, the addition of iodine to the decolorizer will inhibit the dissociation of the iodine dyestuff complex and promote a much more selective differentiation. Experience confirms fully this claim, as is shown by the results in Table I.

Table I

Time of Decolorization of All or Almost All the Bacterial Bodies of Pure-Culture Films

(Method: Violet, 20 sec.; Lugol, 20 sec.; Decolorizer, Alcohol-Acetone 2/1)

	Iodized Decolorizer	Decolorizer without Iodine
B. coli	5 sec.	5 sec.
Staphylococcus	1 hour	5 minutes

Table I shows that, notwithstanding the iodine content of the decolorizer, the differentiation time for the gram-negative bacteria does not change, while use of such iodized decolorizer increases about ten times the resistance of the dyed gram-positive organisms against the decolorization.

The contrast is still more obvious in the case of smears of organic products of irregular thickness, as sputum or vaginal secretion. It is known that the water retained in the smears from the aqueous stains diminishes the selec-

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tivity of the differentiation with absolute alcohol or acetone. The selective action of iodized decolorizers is less affected by moisture than that of the usual pure solvents.

It may therefore be recommended to introduce in the old and in the modified Gram's methods one modification more, that is the addition of a little iodine to the decolorizing solvent or mixture. The amount of iodine to be added need not be very exact. It is sufficient to add drops of *Tinctura iodii* to the solvent until it becomes a light brownish color.

The modification is suitable with any one of the well-known formulas for gram-staining. If acetone or alcohol acetone mixtures are used as decolorizers the iodine must be added at the last moment, because it reacts slowly with acetone if the mixture is stored. It is, therefore, more practicable to choose a staining formula in which absolute alcohol is the decolorizer, since in it the dilute tincture of iodine is stable and can be kept in a drop bottle. Dilute carbol-fuchsin, not safranin, shall be used for the counterstaining, because the yellowish red color of safranin does not contrast as sharply against the greenish black color of the iodine-methyl-violet compound, as the more purplish tone of fuchsin.

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MEASURING CHANGES IN THE INTRACRANIAL PRESSURE OF EXPERIMENTAL ANIMALS*

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IT was found necessary to measure changes in cerebrospinal pressure during sacrifice experiments on cats and dogs. Several methods were tried without any degree of success until the following was suggested by Dr. Reginald A. Cutting. Upon trial, it was found to give excellent results.

A steel rod of ⅜ inch diameter was turned on the lathe to just the size and shape of the trephine to be used (⅜ inch at the top tapering through ¾ inch to ¼ inch at the cutting edge). Above this was turned a nozzle to fit heavy rubber pressure tubing. A ⅛ inch hole was then drilled throughout the length of the rod. The trephine-shaped end was threaded with a ¼-20 thread (Fig. 1).

A water manometer was prepared from heavy Pyrex tubing with a ⅛ inch bore. The reading column was made 80 cm. and the other column 40 cm. long. The manometer was mounted on a wooden panel which was graduated along the reading column in millimeters. The manometer was equipped with a three-way ground glass stopcock in the pressure line leading to the animal (Fig. 2).

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In use the parietal bone of the cranium of the animal is laid bare and a hole drilled with the trephine. This is cleaned thoroughly and an incision is made through the dura. Then with the aid of a pair of pliers the threaded end of the steel plug is screwed tightly into the cranium. With the three-way stopcock completely open, the manometer is connected to the plug with heavy rubber

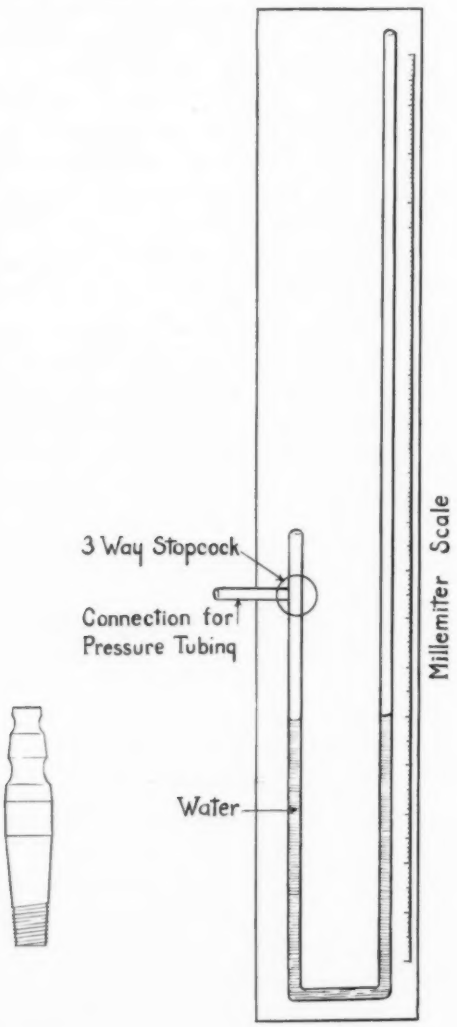

Fig. 1
[Full Size]

Fig. 2

pressure tubing. Care must be taken to insure that all joints are absolutely air-tight. The stopcock is then turned through 45 degrees so that there is an open connection only from the plug to the manometer. A reading of the manometer is immediately taken. This represents the zero point or normal for that animal. Readings showing rise or fall in pressure may then be made at any desired interval during the experiment.

In the case of a rise in pressure the zero point reading is subtracted from the actual reading and the result multiplied by two. In a fall of pressure the actual reading is subtracted from the zero point reading and multiplied by two. In either case the answer is in millimeters of water, positive or negative pressure. To obtain the reading in millimeters of mercury the answer must be divided by 13.6.

THE DISAPPEARANCE OF PHENOLS AND CRESOLS ADDED TO "BIOLOGICAL PRODUCTS" ON STANDING*

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INTRODUCTION

"BIOLOGICAL PRODUCTS" may be said to include vaccines, antiserums, and antiplasmas, and various materials, such as antitoxin preparations, related to or derived from these. Their importance and significance are increasing rapidly both for therapeutic use and (with perhaps more widespread application) for immunization. Unfortunately, the chemical knowledge underlying the products in question lags far behind their practical applications.

In the preparation of these "biological products," their potencies are necessarily of the highest significance. Their value naturally rests upon the requisite physiologic response of the organism. This phase of the subject does not enter into the present problem. Of perhaps greater importance is the safety factor of these products; that no direct harm will ensue from their use. Many of the products are used intravenously, sub- or intracutaneously. All contain protein and, in general, would serve as excellent media for bacterial growths. Such growths may well produce toxins or other harmful products or be themselves a danger if the material containing them were used. One phase of this problem will be taken up here and an experimental investigation of certain actions and relations presented. The results will be limited to vaccines and antitoxin preparations in the present instance, but the methods used, and in all probability the conclusions arrived at, possess general applicability.

The safety factor in connection with a bacterial vaccine or an antitoxin material involves its preparation, storage, and use. The preparation lies outside this presentation. The product, when ready for use, is sterile. To maintain the sterility, a preservative is added. Carbolic acid and the cresols have been perhaps the most commonly used preservatives in amounts up to 0.5 per cent.

In the practical development, the material is prepared, preservative added, and sterility insured. It is then put up in vials or other containers of suitable size, and the sterility tested again.

*From the Department of Preventive Medicine, New York University College of Medicine. Received for publication, June 5, 1936.

Two factors may now enter into the problem. After a certain length of time, definite for each type of product (expiration date), the vaccine, antitoxin (or other product), is returned to the producer, retested for potency, and if found satisfactory and sterile, revialed and redistributed for use. Also, at times, larger vials may be employed and the material of any one vial may not be completely used immediately but only after an interval, as, for example, when immunizing a larger number of individuals, or when one individual is immunized with the same material at intervals.

In these cases, the possibility of contamination of the material exists. Presumably the preservative present will destroy or kill any accidental bacterial contamination. It was observed, however, that products to which 0.45 per cent phenol had been added originally did not contain this amount of phenol after a time. As this raises an extremely important point in connection with the safety factor, a study of this apparent disappearance was undertaken. A number of the results obtained will be presented.

EXPERIMENTAL METHODS

The experimental work to be presented will include the estimation of phenol and cresol in a number of vaccine and antitoxin preparations which had been kept under the customary conditions of storage of biological products.

The significance of the results will depend upon the accuracy of the phenol estimations. The method of analysis consisted in the quantitative formation of tribromphenol by the addition of a bromide-bromate solution and titration of excess bromine with thiosulphate after the addition of potassium iodide, hydrochloric acid, and starch. The method was used first by Koppeschaar,[1] studied further by a number of workers, and a careful reinvestigation given in detail by Scott[2] several years ago.

In view of Scott's extended study and detailed presentation of the method, it will not be repeated here. For the removal of protein, the well-known Folin-Wu precipitation was used. To 5 c.c. of the vaccine or antitoxin, 35 c.c. of water were added, then 5 c.c. of 10 per cent sodium tungstate solution and 5 c.c. of 0.67 N sulphuric acid. The mixture was shaken, allowed to stand at room temperature for thirty minutes, and filtered through paper. To 40 c.c. of the filtrate, 160 c.c. water were added, and the phenol estimation carried out.

Duplicate determinations as a rule agreed within 0.5 per cent of the amount of phenol found. Using the purest phenol obtainable, satisfactory recoveries, within 2 per cent, of the amount added, were obtained.

The results will be given in terms of percentage of phenol present, although in a number of the preparations a bactericidal material containing a mixture of the three cresols was used.

It was shown by Scott that the method of analysis was accurate for the estimation of phenol as stated, but that for the cresols, while the determination could readily be carried out, there was not a quantitative formation of the tribrom derivatives of the ortho and para compounds. Where such a cresol mixture was used in the data to be presented, the results will be calculated and given in terms of phenol. The cresol mixture when analyzed as described was

found to contain material equivalent to 81 per cent calculated as phenol. In other words, a found value of 0.324 per cent calculated as phenol where the cresol mixture was used would correspond to 0.00324/0.81 or 0.4 per cent cresols.

EXPERIMENTAL RESULTS

The results obtained with vaccines will first be presented and then those with a number of antitoxin preparations which contained much higher percentages of protein (10 to 12 per cent as against 4 per cent or less). With the vaccines, in every case (in Table I) 0.45 per cent phenol was added to the vaccine when it was prepared. A number of vaccines were obtained which had been kept in vials with rubber stoppers at ice box temperatures for different lengths of time up to six years. Because of the small quantities of some of the vaccines which were available, different kinds were combined for a number of tests. Those combined and run together had been prepared at practically the same date. These results are presented in Table I.

TABLE I

PHENOL CONTENTS OF VARIOUS VACCINES (CONTAINING ORIGINALLY 0.45 PER CENT PHENOL) AFTER STANDING AT ICE BOX TEMPERATURES THE INDICATED NUMBER OF DAYS

CHARACTER OF VACCINE	NUMBER OF ORGANISMS PER C.C.	TIME OF STANDING (DAYS)	PER CENT PHENOL FOUND
Pertussis	5 billion	1845	0.327
Gonococcus	4 billion	1456	0.293
Cholera	8 billion	1820	0.304
Combined streptococcus	1.2 billion	780	0.331
Typhoid	1 billion	972	0.427
Catarrhalis combined	1.2 billion	174	0.409
Catarrhalis combined	1.2 billion	824	0.355
Catarrhalis influenza	1.4 billion	814	0.277
Catarrhalis influenza	1.4 billion	1830	0.248
Influenza combined	3 billion	1300	0.101
Pertussis Staphylococcus	5 billion 2 billion	10	0.456
Pertussis Staphylococcus	5 billion 2 billion	385	0.380
Pertussis Typhoid	5 billion 1 billion	732	0.340
Typhoid Staphylococcus	1 billion 250 million	2205	0.274
Typhoid Influenza	1 billion 750 million	1100	0.311
Gonococcus Acne	1200 million 440 million	265	0.417

The results in Table I showed marked decreases in the phenol contents after standing. Unfortunately, in this part of the study, it was not practicable to study the same products for the different periods of time. In view of the preliminary or orienting nature of the tests, it is not advisable to attempt to draw general conclusions from them. It may be stated, however, that the numbers of killed organisms did not appear to be the main factor involved.

It seemed possible that the rubber stoppers which were used with the vials might also play a part perhaps by taking up some of the phenol and removing it from the sphere of action. Experiments were therefore carried out with

influenza and typhoid vaccines, at 3 to 5° C. and 37 to 38° C., and with rubber-stoppered and glass-stoppered containers. The results of a series of tests with these two vaccines, each containing 2 billion killed organisms per c.c., and in the presence and absence of rubber stoppers, are presented in Table II. The amount of phenol added was 0.5 per cent, and each vaccine was divided into 3 portions of over 500 c.c. each. One portion (A) was placed in a glass-stoppered bottle after the addition of a number of rubber stoppers to the liquid and kept at 37° C. for 237 days and at 3 to 5° C. for twenty-two days, a second portion (B) was placed in a glass-stoppered bottle and kept at 37° C. for 237 days and at 3 to 5° C. for twenty-two days, and a third portion (C) was placed in a glass-stoppered bottle and kept at 3 to 5° C. for 259 days. The results of the phenol determinations at the end of the time are given in Table II.

These results indicate unmistakably that the rubber stoppers caused the decreases in the phenol contents, that the character of the organism and the temperature of storage played an insignificant or no part in these changes.

TABLE II

PHENOL CONTENTS OF INFLUENZA AND TYPHOID VACCINES (CONTAINING ORIGINALLY 0.50 PER CENT PHENOL) AFTER STANDING IN GLASS-STOPPERED BOTTLES UNDER THE INDICATED CONDITIONS

TREATMENT	TIME AND TEMPERATURE OF STANDING	PER CENT PHENOL FOUND
Influenza Vaccine:		
A. Rubber stoppers present	237 days 37° then 22 days 3-5°	0.390
B. No rubber stoppers	237 days 37° then 22 days 3-5°	0.517
C. No rubber stoppers	259 days 3-5°	0.501
Typhoid Vaccine:		
A. Rubber stoppers present	237 days 37° 22 days 3-5°	0.389
B. No rubber stoppers	237 days 37° 22 days 3-5°	0.511
C. No rubber stoppers	259 days 3-5°	0.497

TABLE III

ANTISEPTIC CONTENTS IN TERMS OF PERCENTAGES OF PHENOL FOUND IN ANTITOXIN PREPARATIONS AFTER STANDING VARIOUS LENGTHS OF TIME

PREPARATION	TYPE OF CONTAINER	PER CENT ADDED		STANDING (DAYS)	FOUND, CALCULATED AS PER CENT PHENOL
		CRESOLS	PHENOL		
C	Vials	0	0.5	0 (Bulk)	0.460
G	Vials	0	0.5	0 (Bulk)	0.511
F	Vials	0	0.5	334	0.513
E	Vials	0	0.5	340	0.380
A	Vials	0.4	0	1040	0.258
R	Syringes	0	0.5	376	0.325
N	Syringes	0.4	0	457, 1036 mixed	0.173
Q	Syringes	0.4	0	775	0.176
L	Syringes	0.4	0	1426	0.174
Z	Vials	0	0.5	77	0.509
X	Vials	0	0.5	475	0.503
Y	Vials	0	0.5	544	0.468

The results obtained with some antitoxin solutions are given in Table III. The times of standing refer to the material in vials or syringes. Five and 10 c.c. containers were used (in a few cases 2 c.c. and 25 c.c.). The vials and syringes were stored in the ice box (5° C.) and tested after the indicated lengths of time. Preparations within the range A to R refer to diphtheria antitoxin, and X to Z to tetanus antitoxin.

The vials were closed with small rubber stoppers, while the syringes in addition to the rubber stoppers contained rubber plungers with considerable surfaces (the width of the containers) exposed to the solutions. The results show that, with phenol in the vials, there was a marked decrease in one case only (Exper. E). The remainder showed no definite decreases. The only experiment with a syringe sample (R) showed a real decrease. In the experiments in which the cresol mixture was used, it must be recalled that 0.4 per cent cresols added correspond to a found value in terms of phenol of 0.324 per cent. The one vial containing cresols (A) showed a value of 0.258 per cent, and the three syringes (N, Q, L) values of 0.173-0.176 per cent. The lengths of times of standing in all cases where marked decreases were shown were more than a year.

DISCUSSION

In the first place, the accuracy of the phenol estimations is sufficiently great to make it possible to draw definite conclusions even for changes considerably less than those presented here. It may be stated that estimations of phenol contents of these products made in a different laboratory agreed surprisingly closely with the results found in this laboratory. The decreases in the phenol contents may therefore be accepted as established.

The question of the safety of these and other products handled similarly must be considered. Just what percentages of phenol (or of cresols where these were used) constitutes "safety" cannot be stated arbitrarily. While 0.5 per cent seems to allow a fairly wide margin, much may depend upon the special organism present. Data on these questions are being collected elsewhere and will be presented in due course.[3]

The fact that the rubber stoppers are the cause of the disappearance of the phenol from the vaccine and the antitoxin preparations raises a number of questions, such as the most satisfactory type of stopper, the possibility of obtaining phenol resistant stoppers, etc., which lie beyond the scope of this paper.

The character of the combination of rubber and phenol was not investigated.

In the practical use of vaccines, antitoxins, etc., it is most desirable, if not essential, to determine the phenol content at definite intervals.

Although the results presented in this paper are limited to vaccines and antitoxins, it is evident that any biological product containing phenol (or cresols) as preservative and stored in rubber-stoppered containers, is open to suspicion.

SUMMARY

Phenol, used as a preservative in maintaining the sterility of vaccines and antitoxins, was found to decrease in amount when these products were stored in rubber-stoppered vials, or in the presence of rubber stoppers. The rubber was found to remove the phenol from the vaccine.

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THE NUMERICAL DETERMINATION OF TRICHOMONAS HOMINIS IN URINE AND ITS PRACTICAL IMPLICATIONS IN GENITOURINARY PARASITISM*

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RECENTLY, in demonstrating a clinical problem to students, a voided specimen of urine from a male was found to contain trichomonads, presumably *Trichomonas vaginalis* Donné, 1837. In discussing the degree of involvement it was pointed out that this, probably, would have to be determined upon mere impression gained from the microscopic examination of varying sized droplets. However, the knowledge derived in the establishing of a method for the numerical determination of protozoa in vitro, which was modified to apply clinically in two cases of human intestinal parasitic involvement,[1] was used to count the trichomonads present in this voided urine as follows:

Seven tubes, each containing 5 c.c. of the well-mixed specimen, were treated in the identical manner as were *Trichomonas hominis* cultures in the study referred to above, except that 1 per cent formalin, found to serve the same purpose just as effectively, was used instead of 10 per cent. The experiences with this urine appeared to be identical with those of the cultural studies. The organisms were found, as occasionally occurred in our cultures, to be too motile for accurate hemocytometric counting; then a drop or two of 1 per cent formalin placed in the urine resulted in nonmotile, apparently fixed, hyaline-like and, as far as could be determined, nondisintegrated trichomonads. The organisms were counted by the hemocytometer as are cells in spinal fluid. This method of counting needs no further description here. The high dry power lens and a mechanical stage were used in these determinations, thereby facilitating the procedure and increasing accuracy.

The number of trichomonads per cubic millimeter of voided urine was as follows:

Test Tube 1	8 Trichomonads
Test Tube 2	9 Trichomonads
Test Tube 3	9 Trichomonads
Test Tube 4	10 Trichomonads

Next, the hemocytometer containing other samples of this urine untreated with formalin was inverted for a few minutes over the open mouth of a bottle

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containing a 40 per cent formalin solution, the cover slip being attached only on two sides by petrolatum. The trichomonads per cubic millimeter were after some minutes immobilized.

Test Tube 5	9 Trichomonads
Test Tube 6	8 Trichomonads
Test Tube 7	9 Trichomonads

The addition of two drops of 1 per cent formalin to a 5 c.c. sample of urine, thus to be examined, was found to be simpler and therefore preferable (as similarly experienced in our culture studies) to the exposure of untreated urine to formalin fumes. Later, it was learned that as little as two drops of 0.25 per cent formalin to the urine or culture frequently accomplished the same result.

Finally, Tubes 1, 2, 3, and 4 were centrifuged for five minutes at 3,000 revolutions per minute. Four cubic centimeters of the supernatant fluid was completely removed by a capillary pipette, and the hemocytometer was filled with the mixed residue or sediment. The apparently fixed, hyaline-like trichomonads were readily visible and easily counted. No evidence of any destruction of the organisms was encountered. The number of organisms per cubic millimeter of residue of voided urine was as follows:

Test Tube 1	40
Test Tube 2	41
Test Tube 3	49
Test Tube 4	40

These studies are not as extensive as one would wish, but such material as is necessary for further investigation has been encountered rarely. In order to repeat the above experiments in so far as possible, the trichomonads in urine were simulated by the addition of 0.25 c.c. of a well-mixed culture of *Trichomonas hominis* in Andrews' modification of serum-saline-citrate medium[2] to 4.75 c.c. of urine, such urine having been previously determined as being normal in all respects. This was incubated overnight. In the morning the trichomonads were found to be actively motile. To each tube of 5 c.c. of this trichomonad-infested urine was added two drops of 1 per cent solution of formalin. The hemocytometer was then filled, and the results were as follows:

Tube 1:	First count	31
	Second count, another sample	35
Tube 2:	First count	35
	Second count, another sample	26
	Third count, another sample	34

To determine the effect of centrifugalization on trichomonads, this urine was then centrifugalized at about 3,000 revolutions per minute for five minutes. Four cubic centimeters of the supernatant fluid was removed. This contained no demonstrable organisms. The residue and the remaining supernatant fluid were mixed with a capillary pipette and the number of organisms was then determined by the hemocytometer method as indicated, with results as follows:

Tube 1:	First count	118
	Second count, another sample	123
Tube 2:	First count	141
	Second count, another sample	134

The 4 c.c. of supernatant fluid which was removed from each of these tubes was then replaced, the material mixed thoroughly and the counts of the two tubes made as follows:

Tube 1:	29
Tube 2:	36

This was similarly repeated with identical methods, using another strain of *Trichomonas hominis* at a later date, with the following results:

Tube A:	First count	50
	Second count, another sample	46
Tube B:	First count	35
	Second count, second sample	38

After centrifugalization and removing 4 c.c. of the supernatant fluid, as noted, the remaining material, mixed thoroughly, gave these counts:

Tube A:	First count	262
	Second count, second sample	252
Tube B:	First count	221
	Second count, second sample	220

When the 4 c.c. of the supernatant fluid which had been drawn after centrifugalization was replaced in its respective tube, and then well mixed with the residue, the following counts were obtained:

Tube A:	54
Tube B:	47

COMMENT

The closeness of these counts is obvious; hence the relative accuracy of the hemocytometer for practical purposes in the counting of trichomonads in urine needs no further comment. Incidentally, the coefficient of variability in the use of the hemocytometer in the numerical determinations of *E. histolytica* in vitro was found by me to be under 5 per cent.[1] It is interesting to note that Hegner[3] and his students first used the hemocytometer in trichomonad studies in lower animals. As far as can be determined, this communication is its first recorded clinical application.

Our studies indicate that trichomonads in urine, treated with formalin, are not destroyed when submitted subsequently to centrifugalization. Identical results from a limited number of *Trichomonas hominis* serum-saline-citrate cultures when submitted to this identical procedure would tend further to confirm this.

It seems possible to determine quantitatively the degree of urinary trichomoniasis by the numerical determination of the protozoan in the manner described above. Thus, progression of the condition and the efficacy of therapy can better be evaluated. No cases of vaginal trichomoniasis have been available for investigation by this method. However, it is suggested that a quantitative determination could be made by treating the returns of a douche of a pint or quart of saline in the same manner as noted for trichomonads encountered in urine. Trichomonads in prostatic secretion can also be determined by the

hemocytometer. When the organisms are too active for accurate counting, the chamber containing the material may be exposed to the fumes of formalin by inverting it over the mouth of a bottle containing a 40 per cent solution, the cover slip being attached only on two sides by petrolatum, thus fixing the organisms.

The ability to centrifuge samples either of urine containing this organism or of the returns of a douche in vaginal trichomoniasis should aid in detecting mild or subsiding involvements which otherwise might appear to be negative. In addition, there is nothing in the morphologic and biologic characteristics of trophozoite forms of other flagellates which would prevent the satisfactory application of this method if and when they are detected in the type of material here noted. It would seem that the number of vegetative forms of amebae and ciliates, as well as cysts of all protozoa (except for trichomonas cysts of which so far none have been demonstrated), could be counted similarly, but without the exhibition of formalin since the degree of motility of these forms would not interfere with the procedure of counting.

Finally, the great dilution of formalin which appears to be toxic to trichomonads suggests its possible use in the therapy of trichomonad infection or infestation, both in the urinary and digestive tracts.

SUMMARY

1. It is possible now to determine quantitatively the degree of urinary trichomoniasis by the use of the hemocytometer. The flagellates are counted as are cells in spinal fluid and the number per cubic millimeter of the sample examined is thus obtained. When the protozoan is too motile for accurate enumeration, two drops either of 0.25 per cent formalin or of 1 per cent formalin are added to 5 c.c. of the material. Centrifugalization without apparent injury to the trichomonads is possible, especially if formalin is used as indicated prior to this procedure.

2. The application of this method in vaginal trichomoniasis, as well as a modification of it outlined in the text for the numerical determination of these organisms in prostatic secretion, has been suggested.

3. The clinical significance of this procedure rests in the ability to estimate quantitatively the extent of infestation, its progression or decline, and the efficacy of therapy.

4. There is nothing in the morphologic and biologic characteristics of other flagellates, amebae and ciliates, if and when so encountered, that would prevent a satisfactory application of this method.

5. The great dilution of formalin which appears to be toxic to trichomonads suggests its possible use in therapy, both in the urinary and digestive tracts.

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MEDICAL ARTS BUILDING

HORMONE MEDIA AS A BASIS FOR FUNGUS CULTURES*

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AFTER having accidentally obtained several fungus growths upon routine blood hormone agar slants from unsuspected cases, we began to experiment with hormone media, basing our efforts upon the premise that the beef heart hormones facilitated the growth of fungi just as they did that of the bacteria.

This work has been carried on as a part of the routine of the laboratory, covering a period of six months; hence, the variety of cultures used has been limited necessarily to those obtained from patients in the hospital during that time. By using the beef heart infusion medium, we have been able to uncover more fungus infections than we had succeeded in doing previously in an equal period.

In our experiments, the basis of the media used was heart infusion broth, made after a modified formula of Huntoon. This was used in several variations as seen in Table I.

Since Sabouraud's medium is slightly acid, pH 5.7, the hormone medium was reduced from its natural alkalinity of pH 7.3 to pH 5.7, and parallel cultures of several strains of fungi (actinomyces, coccidioides, monilia, epidermophyton) were inoculated upon slants having the two reactions. No perceptible difference in rapidity or volume of growth could be noted.

In obtaining material from the patients it was found advisable to eliminate contaminating bacteria as far as possible by the thorough sterilization of the field surrounding the lesion and observing the most careful technic, as even the acid version of this medium does not inhibit bacterial growth as does Sabouraud's.

The highest percentage of positives was obtained when several types of the media were inoculated, and incubation was continued for several weeks. One strain of actinomyces showed a definite preference for the potato slant. A beautiful play of colors was obtained upon this culture; from the original white the whole slant became a deep indigo in two weeks, and later fringes of coral began to appear. Another strain of actinomyces grew only in the broth made anaerobic with mineral oil, and in deep agar stabs, 3 cm. from the surface. A characteristic black growth began to appear in three days. The potato slant and broth proved quite unsatisfactory, however, for an epidermophyton obtained

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TABLE I

SPECIES OF FUNGUS

MEDIUM USED			ACTINOMYCES (AEROBIC STRAIN)	ACTINOMYCES (ANAEROBIC STRAIN)	COCCIDIOIDES	EPIDERMOPHYTON	MONILIA
3.2% HEART INFUSION BROTH							
pH 7.3	1% Dextrose				Luxuriant growth		Luxuriant growth
pH 7.3	1% Dextrose layer of mineral oil			Growth at bottom of tube			
pH 7.3	1% Dextrose saturating cotton surmounted by potato slant		Luxuriant growth		Luxuriant growth	Moderate growth	
pH 7.3	Solidified with 2% filtered agar (hormone media may not be filtered)	0.1% Dextrose slant	Good growth	Growth 3 cm. from surface	Good growth	Good growth	Good growth
pH 7.3		0.5% Dextrose slant	Good growth		Good growth	Good growth	Good growth
pH 7.3		1.0% Dextrose slant	Good growth		Luxuriant growth	Luxuriant growth	Luxuriant growth
pH 7.3		Stab					
pH 5.7		0.1% Dextrose slant	Good growth		Good growth	Good growth	Good growth
pH 5.7		0.5% Dextrose slant	Good growth		Good growth	Good growth	Good growth
pH 5.7		1.0% Dextrose slant	Good growth		Luxuriant growth	Luxuriant growth	Good growth

The anaerobic strain of actinomyces, very difficult of culture, died out before completion of the experiment. Stab cultures were attempted but not obtained upon the various sugar contents.

from a so-called "athlete's foot," but a luxuriant growth appeared after three weeks on the agar slants. The monilia thrived impartially upon all the aerobic types. Several excellent cultures of coccidioides were obtained upon agar slants, although they grew equally well in broth. These develop rapidly even on the blood slants used routinely for bacterial cultures, a white mycelial growth being observed in two or three days, which rapidly becomes luxuriant a few days after transplanting to the dextrose slants.

CONCLUSIONS

The use of beef heart infusion as a basis for culture media for fungi is highly satisfactory. Since modifying the pH does not inhibit the bacterial growth, and the fungi grow luxuriantly in the alkaline medium, and since a 1 per cent dextrose seems to be slightly more favorable to growth than a lesser amount, it is concluded that this percentage in the natural alkaline medium is the simplest and most satisfactory to use.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MENINGOCOCCUS MENINGITIS, The Control of Epidemics by Active Immunization With Meningococcus Soluble Toxin, Kuhns, D. M. J. A. M. A. 107: 5, 1936.

An epidemic of meningococcic meningitis occurred in which there were seventeen cases.

a. The greatest number of cases occurred where there was the greatest density of population, in a CCC camp near a small town.

b. The mortality in the epidemic was 83 per cent in six untreated patients outside the CCC camp and 11 per cent in nine patients treated early with antimeningococcus serum.

A culture for carriers was made in the CCC camp, Camp Crystal, on four successive weeks and the highest number found positive at one time was 4.4 per cent. A culture was made at the same time on a nonepidemic camp 20 miles distant and 3.5 per cent were positive.

On another cultural study made by Ross Laybourn at Camp Coffey, 3.5 per cent were found positive.

It was noted that cases continued to occur in the CCC camps after all the control measures available at the present time were enforced. The control measure considered to be of the greatest value was the separation of individuals the greatest distance possible from one another.

A study of filter separable fractions of meningococcus broth culture was made to determine the skin reactive properties of Gordon's four types of meningococcus.

A. It was found that the whole culture produced the greatest reaction and the filtrate produced the next greatest reaction.

B. The heated filtrate produced no reaction and served as a control.

C. The filtrate from autolyzed meningococci, or endotoxin, produced no skin reaction.

The filtrate was given a trial in an epidemic of meningitis to determine its value as a skin testing and immunizing substance.

A new approach as to how to determine the level of immunity of a susceptible individual by skin testing with graduated dilutions of filtrate on an individual coming down with the disease and again after recovery is suggested as a possible method of determining the skin test unit dose.

a. The amount of filtrate necessary to change the skin test from positive to negative should be the amount necessary to immunize the individual.

b. The skin test reaction could be a very valuable guide in the treatment of the individual.

The filtrate was used in a dilution of 1:100 as a skin-testing substance on two- and four-week convalescents and gave consistently negative tests.

Skin testing on sensitive and susceptible individuals is the only available means at the present time of determining the toxicity of the exotoxin. Although the laboratory animals do react, the effects are not sufficiently consistent to titrate the potency of the product.

A 1:100 dilution of a filtrate produced from Types 1, 2, 3, and 4 meningococcus was used to make skin tests of 2,000 newly enrolled CCC members; 37 per cent gave a skin reaction of one-plus or greater.

Although the filtrate that was used in the Tarkio epidemic did not appear as stable as the one the authors are developing at the present time, the skin test there indicates a trend from positive to negative.

A filtrate aged 9 months in a refrigerator held at 50° C. reacts on 10 per cent less of the same enrollees than one made nine months later.

An opportunity to observe the change in skin tests after subcutaneous inoculation with full strength filtrate, after an interval of four months, was offered at Camp Coffey and the trend was definitely from positive to negative.

Among 395 individuals receiving meningococcus filtrate subcutaneously, none have developed meningitis, even though in the Tarkio epidemic four cases developed among those not immunized. Ten months have intervened since this immunization was first initiated.

The filtrate has been used in three outbreaks not reported in this paper with the same results as described.

It is to be remembered that this is only a preliminary report and in order to prove the value of the meningococcus filtrate as a skin-testing and immunizing agent it should be used in a large number of beginning epidemics and over a long period of time.

RENAL FUNCTION: The Excretion of Ferrocyanide in Man in Relation to the Urea Clearance, Gordon, W. Am. J. M. Sc. **192**: 208, 1936.

The ferrocyanide excretion test of renal function was compared with the simultaneous urea clearance in 102 subjects with a range of clearance levels from 6 to 130 per cent. Following intravenous injection of 0.28 gm. of sodium ferrocyanide, normal subjects excreted at least 24 per cent in one hour, 35 per cent in two hours, and 50 per cent in three hours. There was a correlation of about 0.7 between urea clearance and ferrocyanide excretion for each of these three periods. One hour proved to be an adequate length of time for the test. The administration of ferrocyanide did not alter the clearance. In those instances in which the tests gave discordant results other evidence pointed to error in each of the two methods with about equal frequency. The physiologic significance of the test is discussed. Considerable value in detection of impairment of renal function may be expected of the ferrocyanide test, but its value in observing the progress of renal disease cannot be judged yet and is somewhat more uncertain. The test is simple and harmless in most instances. Great care is required in estimating ferrocyanide in the presence of hematuria and some accuracy is sacrificed if much blood is present in the urine. Patients with prostatic hypertrophy had dysuria and inability to void in some instances.

UNDULANT FEVER, Treatment of, by the Intravenous Injection of Killed Typhoid, Paratyphoid A and Paratyphoid B. Bacilli, Ervin, C. E., Hunt, H. F., and Niles, J. S. Am. J. M. Sc. **192**: 234, 1936.

Of 12 cases of undulant fever observed, 10 were treated by the intravenous administration of typhoid vaccine. The results in all 10 were satisfactory. There have been no recurrences and the follow-up agglutination tests for *Br. abortus* were negative in 9 of the 10 patients treated. Two follow-up agglutinations in control cases were also negative.

Those results compare favorably with the treatment reported by other authors who have used specific substances prepared from *Br. abortus*.

The distinct advantages of typhoid vaccine are its availability in all localities and the simplicity with which it can be administered.

TUMORS, Cytological Studies of Malignant, Von Haam, E., and Alexander, H. G. Am. J. Clin. Path. **6**: 394, 1936.

By means of a modified biometric method, the nuclear-nucleolar ratio was determined in 10,000 cells chosen at random from fifty malignant tumors and fifty benign tissues.

A comparison of average values, obtained from groups of one hundred cells examined in each specimen, demonstrates that 96 per cent of the malignant lesions showed a nuclear-nucleolar ratio distinctly lower than that found in the benign tissues.

The corpus luteum cells of the ovary are likewise characterized by a very low nuclear-nucleolar ratio.

The study of the nucleoli is emphasized as valuable help in the tumor diagnosis, although none of the changes reported represents a criterion absolutely characteristic for each malignant cell.

Because of lack of knowledge concerning the biological functions of the nucleolus, the authors can, at the present time, offer no interpretation of their observation.

LEAD POISONING, Ratio of Large to Small Lymphocytes in, Shiels, D. O. Med. J. Australia **1**: 847, 1936.

Absorption of lead causes an increase in the ratio of large lymphocytes plus monocytes to small lymphocytes.

A fall in this ratio below 2:1 while the subject is exposed to the hazard is associated with definite symptoms of lead poisoning, usually of sufficiently severe nature to cause incapacity.

Generally speaking, the more severe the case, the lower the ratio.

The magnitude of this ratio is more closely associated with the clinical condition than is the stippled cell count.

The magnitude of this ratio is a simple and very useful indication by which to judge of the imminence or otherwise of lead poisoning and is an aid to diagnosis.

LYMPHOGRANULOMA, Use of Mouse Brain Antigens for Diagnosis of, Grace, A. W., and Suskind, F. H. Arch. Dermat. & Syph. **34**: 65, 1936.

It has been found that Frei antigen can be prepared from the brain of a mouse infected with the virus of lymphogranuloma inguinale.

The original Frei antigen as prepared from pus from a human being has many drawbacks.

There are at least four distinct conditions due to lymphogranuloma inguinale which may be diagnosed by the performance of the Frei test.

Antigen prepared from mouse brain has been tried for one year at the New York Hospital for the diagnosis of lymphogranuloma inguinale. The preparation and administration of 88 different specimens of antigen, derived from the brains of mice used for the second to the forty-fourth passage of a single strain of virus, and the results of their use in 27 patients with the disease and in 38 nonlymphogranulomatous subjects are described in detail.

The average positive reaction to antigen prepared from mouse brain was an erythematous papule 8.7 mm. in diameter, with a surrounding flare of varying size; 77 per cent of these reactions showed a central papule from 7 to 10 mm. in diameter.

No reaction which could be regarded as positive was obtained in any person by the use of antigen prepared from lymphogranulomatous mouse brain in nonlymphogranulomatous persons or by the use of emulsions of normal mouse brain, prepared in the same way as the antigen.

The results of tests on patients with conditions which must be differentiated from lymphogranuloma inguinale were definitely negative.

The presence of active or arrested syphilis had no effect on the reaction to the Frei test performed with antigen prepared from mouse brain.

By standardization of dilution and dosage it was possible to produce reactions quantitatively the same as those to antigens prepared from human pus; qualitatively, however, the reactions were usually more intense.

No desensitization of the skin of any lymphogranulomatous subject was observed after repeated inoculations with antigen prepared from mouse brain. Low grade sensitization to the protein of mouse brain, however, was seen in one instance.

Antigen prepared from mouse brain has been found to retain its potency for at least eight months after preparation.

Experiments have been performed to detect the coexistence of spontaneous virus disease of mice with our strain of the virus of lymphogranuloma inguinale. Only negative results have been obtained.

It is felt, therefore, that antigen prepared from lymphogranulomatous mouse brain can replace that prepared from pus from a human being for the diagnosis of lymphogranuloma inguinale. Not only is it as sensitive and specific as the most potent antigen prepared from human material, but it overcomes the disadvantages of the latter.

LANTERN SLIDES, Simple and Inexpensive Method of Making, Levin, S. J. J. A. M. A. **107**: 124, 1936.

A sheet of cellophane, the size of a standard lantern slide, is placed between two sheets of heavy carbon paper, the carbon side toward the cellophane. These are then placed between two sheets of thin white paper, folded into an envelope of the same size. The material to appear on the slide is typed in the usual manner, a three-fourths inch blank border being left. A clear carbon copy is thus produced on the cellophane, which is then removed and mounted between two lantern slide cover slips, the edges being taped with standard lantern slide binding tape.

Free-hand sketches can be made directly on the cellophane with India ink by means of a fine penpoint. Diagrams of apparatus can be traced in the same fashion. The India ink dries rapidly, after which the sheet of cellophane can be mounted as described.

One of the chief advantages of this method, aside from the considerable saving involved, is the fact that the images projected on the screen from these slides are much larger than those from lantern slides made by photographic reduction. The amount of material that can be typed on the sheet of cellophane is necessarily limited. This may make it necessary to break up large tables into a number of smaller ones, but the resulting images can be seen even in the rear of large lecture rooms.

BLOOD IODINE, Clinical Experiences in the Use of Determinations of, McCullogh, E. P., and McCullogh, D. R. Arch. Int. Med. **57**: 1061, 1936.

From a study of these cases, it is indicated that in Cleveland the blood iodine levels of normal adults and of patients who have diseases which are not associated with the thyroid gland range from approximately 8 to 12 micrograms per hundred cubic centimeters.

The blood iodine level is very high in cases in which iodine medication has been instituted. Exercise has little influence in raising the level of blood iodine, but strenuous exertion may have a temporarily depressing effect.

Under controlled conditions the blood iodine level is proportionate to thyroid activity in most cases, the change in the level, however, being relatively greater in persons with hyperthyroidism than in those with hypothyroidism.

Determinations of blood iodine are of value in the differential diagnosis of hyperthyroidism.

The method used was devised by one of the authors (McCullogh, D. R.: J. Biol. Chem. **107**: 35, 1934).